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1,2,3-Triazolo[1,5-a]quinoxalines: synthesis and binding to benzodiazepine and adenosine receptors

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Abstract – This paper reports the synthesis and binding assays toward benzodiazepine and adenosine A_1 and A_{2A} receptors of new 1,2,3-triazolo[1,5-a]quinoxalin-4-one derivatives. The prepared compounds show good affinity toward the benzodiazepine receptor (K_i 53–314 nM); the GABA ratio values suggest an inverse agonist activity for the N(5) unsubstituted compounds **6b–d** and an agonist activity for the N(5) methylated compounds **7a–c**. Some derivatives of both series show good affinity (K_i < 100 nM) and selectivity toward the adenosine A_1 receptorial subtype. © Elsevier, Paris

1,2,3-triazolo[1,5-a]quinoxalines / benzodiazepine receptor binding / adenosine receptor binding

1. Introduction

In two previous papers we have reported the synthesis and benzodiazepine receptor binding of one 1,2,3-triazolo[1,5-a][1,3]benzodiazepine series [1] (formula **A**, *figure 1*) and of an analogous 1,2,3-triazolo[1,5-a]quinazoline series [2] (formula **B**, *figure 1*). Some compounds showed an interesting partial-agonist activity and theoretical calculations based upon the structure–activity relationships suggested the introduction of a substituent which generates a negative MEP region on the benzene ring for both of the series.

These tricyclic structures also underwent binding assays toward adenosine A_1 and A_{2A} receptors and the results indicated that some triazoloquinazoline derivatives possess good affinity and selectivity to the A_1 receptorial subtype; the best R substituent in this case appeared to be phenyl, while for the benzodiazepine receptor the ethoxycarbonyl group was preferred [2].

Research about these nitrogenized tricyclic heterocycles was then continued with the two-fold purpose of producing compounds more effective toward either the benzodiazepine or adenosine receptor binding, by introducing substituents on the phenyl ring as well as displacement of the nitrogen atom in the 4 position of the central ring, to form isomeric heterocyclic structures.

In this paper we report the study of new tricyclic compounds bearing a six-membered central ring, by the synthesis and biological evaluation of 1,2,3-triazolo[1,5-a]quinoxalines corresponding to the general formula **C** (*figure 1*). This heterocyclic system had been studied essentially from a chemical point of view [3–7], while its biological properties are unknown.

2. Chemistry

The synthesis of triazoloquinoxaline compounds (*figure 2*) was accomplished starting from 2-nitrophenyl azides, by a 1,3-dipolar cycloaddition reaction to diethyl oxalacetate sodium salt in anhydrous ethanol at ~55 °C for some hours. This reaction provided the expected diethyl-1-(2-nitrophenyl)-1,2,3-triazole-4,5-dicarboxylate **1** in moderate yield (20–30%) because it was formed together with variable amounts of byproducts, mainly 4-ethoxycarbonyl-5-carboxy-1,2,3-triazole **2**, corresponding decarboxylated compound 4-ethoxycarbonyl-1,2,3-triazole **3**, benzoxadiazole-N-oxide **4** and anilines **5**, which was separated by flash-chromatography.

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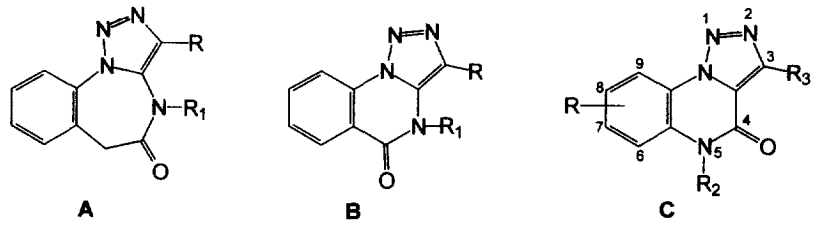
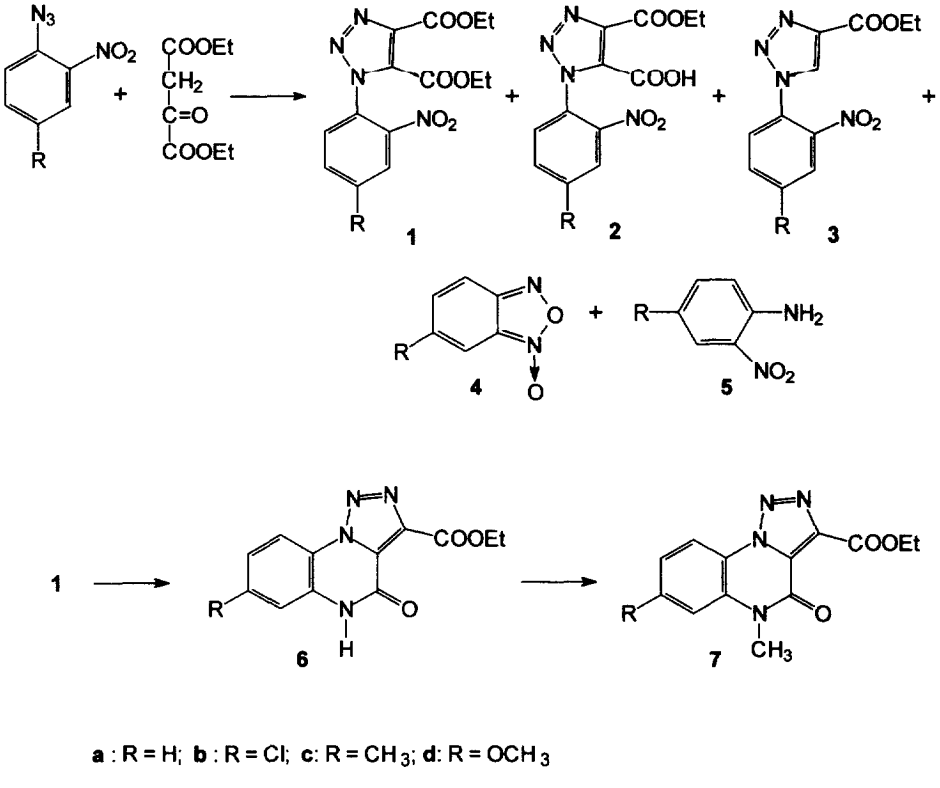


Figure 1. General formulas **A**, **B** and **C**.

Reduction of the nitrophenyltriazoles **1a,c,d** by catalytic hydrogenation (5% Pd/C) gave directly the tricyclic compounds **6a,c,d** coming from the intramolecular condensation of the amino group with the ethoxycarbonyl function in the 5 position; the chloro-substituted compound **1b** needed a chemical reduction with iron powder to prevent hydrogenolysis of the chloro atom (*table I*). Alkylation of compounds **6** with dimethylsulphate in the presence of anhydrous potassium carbonate provided the corresponding *N*-methyl derivatives **7a–d**, as shown by spectroscopic data (*table I*).

The 4-chloro-2-nitro-phenylazide [8] reacted with ethyl propiolate in DMF solution (*figure 3*) to give in 75% yield a mixture of triazole isomers, 4-ethoxycarbonyl-substituted **3b** and 5-ethoxycarbonyl-substituted **8**, isolated by flash chromatography in a ratio ~3:1 respectively. The reduction of these nitrophenyltria-



a : R = H; **b** : R = Cl; **c**: R = CH_3; **d**: R = OCH_3

Figure 2. Synthetic route for compounds **6** and **7**.

Table I. Physical constants, yields and elemental analyses of compounds **6**, **7**, **10**, **11**.

Compound	Yield (%)	M.p. (°C) solvent	Molecular formula (molecular weight)
6a	98	216–220[a]	$C_{12}H_{10}N_4O_3$ (258.24)
6b	98	245–247[a]	$C_{12}H_9N_4O_3Cl$ (292.69)
6c	98	242–245[a]	$C_{13}H_{12}N_4O_3$ (272.26)
6d	98	220–224[a]	$C_{13}H_{12}N_4O_4$ (288.27)
7a	86	173–175[a]	$C_{13}H_{12}N_4O_3$ (272.26)
7b	92	183–186[a]	$C_{13}H_{11}N_4O_3Cl$ (306.71)
7c	68	163–165[b]	$C_{14}H_{14}N_4O_3$ (286.29)
7d	76	185–190[b]	$C_{14}H_{14}N_4O_4$ (302.29)
10a	80	293–296 dec.[b]	$C_9H_6N_4O$ (186.17)
10b	95	273–277 dec.[a]	$C_9H_5N_4OCl$ (220.62)
11a	81	306–309 dec.[c]	$C_{10}H_6N_4O_3$ (230.18)
11b	78	293–297 dec.[b]	$C_{10}H_5N_4O_3Cl$ (264.63)

Crystallization solvent: [a]ethanol; [b]aqueous dimethylformamide; [c]dimethylformamide.

zole isomers provided 1-(2-amino-4-chloro-phenyl)-4-ethoxycarbonyl-1,2,3-triazole **9b** and 7-chloro-1,2,3-triazolo[1,5-a]quinoxalin-4-one **10b** respectively. The same compound **10b** was also obtained from triazoloquinoxaline ester **6b** by hydrolysis to the corresponding acid **11b** and then decarboxylation. Similarly hydrolysis of **6a** gave the acid **11a** which was decarboxylated to **10a**.

The structures of all the new prepared compounds were assigned according to reaction mechanisms and were confirmed by analytical and spectroscopic data: ^{1}H-NMR, ^{13}C-NMR, mass and IR data of the triazoloquinoxalines are reported in *tables II*, *III* and *IV*. The structure assignment of the byproducts obtained by the reaction between 2-nitro-phenylazides and diethyl oxalacetate, i.e. the semiesters **2a–d**, isolated as an acid fraction, and the monoesters **3a–d**, was based upon the characterization of the monoester **3b**. In fact

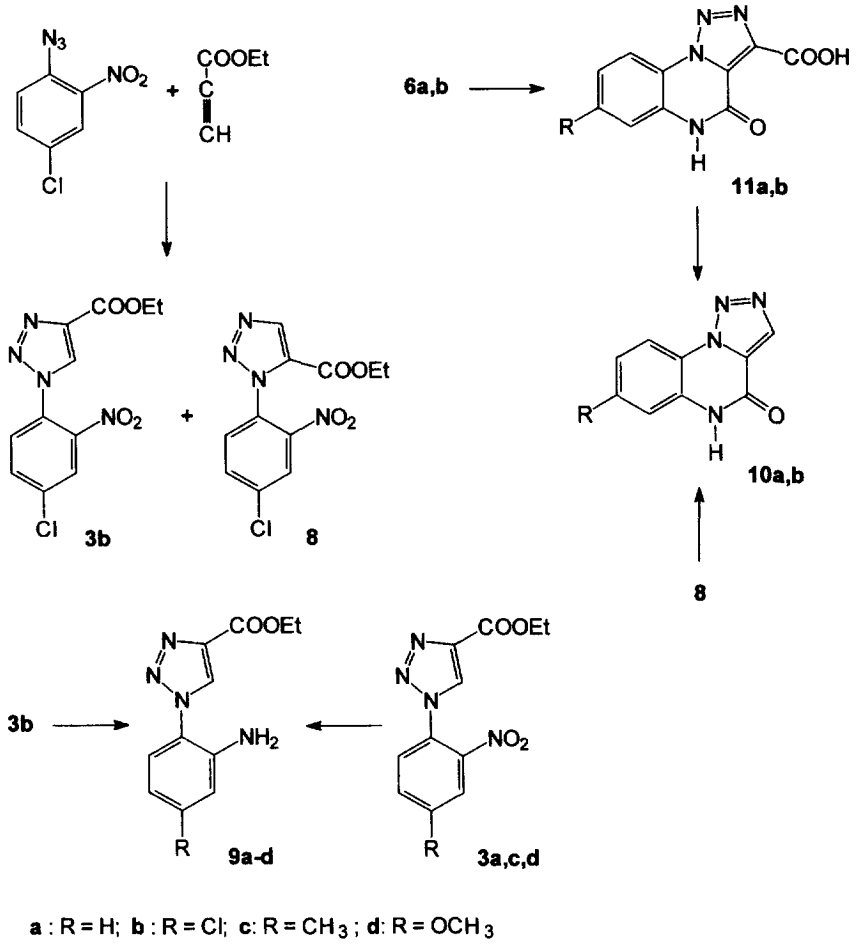


Figure 3. Synthetic route for compounds **9**, **10** and **11**.

Table II. ^{1}H-NMR data (δ: ppm, *J*: Hz) for compounds **6**, **7**, **10**, and **11** in DMSO.

	6a	**6b**	**6c**	**6d**	**7a**	**7b**	**7c**	**7d**	**10a**	**10b**	**11a**	**11b**
H-3	–	–	–	–	–	–	–	–	8.58	8.60	–	–
H-6	7.50	7.45	7.15	6.92	7.65	7.81	7.49	7.10	7.46	7.46	7.57	7.54
H-7	7.56	–	–	–	7.67	–	–	–	7.56	–	7.67	–
H-8	7.43	7.41	7.10	6.95	7.45	7.53	7.26	7.06	7.39	7.42	7.51	7.51
H-9	8.34	8.34	8.11	8.22	8.41	8.46	8.28	8.33	8.33	8.32	8.43	8.42
CH_2	4.42	4.42	4.41	4.41	4.42	4.43	4.41	4.42	–	–	–	–
CH_3	1.37	1.37	1.37	1.37	1.38	1.38	1.38	1.37	–	–	–	–
R-7	–	–	2.36	3.83	–	–	2.48	3.93	–	–	–	–
N-CH_3	–	–	–	–	3.63	3.63	3.61	3.62	–	–	–	–
$J_{6,7}$	n.d.	–	–	–	n.d.	–	–	–	8.24	–	8.30	–
$J_{6,8}$	n.d.	2.16	0.90	2.59	n.d.	2.05	0.94	2.46	1.64	2.22	1.64	2.28
$J_{7,8}$	n.d.	–	–	–	n.d.	–	–	–	6.97	–	6.97	–
$J_{7,9}$	n.d.	–	–	–	n.d.	–	–	–	1.42	–	1.41	–
$J_{8,9}$	n.d.	8.65	8.28	8.68	n.d.	8.74	8.34	8.81	8.13	8.54	8.15	9.18
J_{Et}	7.10	7.09	7.08	7.07	7.10	7.09	7.10	7.10	–	–	–	–

Table III. ^{13}C-NMR data (δ, ppm) for compounds **6**, **7**, **10**, and **11** in DMSO.

	6a	**6b**	**6c**	**6d**	**7a**	**7b**	**7c**	**7d**	**10a**	**10b**	**11a**	**11b**
C-3	137.9	137.9	137.8	137.7	138.0	138.2	138.0	138.1	133.1	133.6	138.1	138.4
C-3a	127.1	126.4	126.2	125.8	125.8	126.0	125.4	125.1	127.5	127.7	126.4	126.1
C-4	152.0	151.8	151.7	151.8	151.3	151.8	152.0	151.6	153.3	153.6	155.1	154.6
C-5a	127.2	130.7	129.4	130.6	128.0	132.0	130.3	131.8	128.9	130.5	128.6	130.1
C-6	116.1	115.8	115.4	100.1	115.9	116.5	115.8	101.1	115.5	116.2	115.8	116.5
C-7	129.8	133.5	139.6	159.5	129.7	134.5	140.3	159.9	129.0	133.1	129.8	133.7
C-8	123.7	123.1	124.3	110.6	123.7	123.9	124.7	110.2	123.4	123.5	124.7	124.3
C-9	116.6	117.5	116.1	117.0	116.2	117.9	116.4	117.7	116.7	117.5	117.4	117.6
C-9a	120.2	119.4	118.2	114.5	120.7	120.1	118.8	114.8	120.7	120.0	121.0	119.9
COO	159.8	159.4	159.6	159.9	159.5	159.8	159.8	160.3	–	–	158.6	158.9
CH_2	61.5	61.2	61.1	61.0	61.2	61.7	61.5	61.1	–	–	–	–
CH_3	14.1	13.8	13.8	13.8	13.7	14.1	14.1	13.8	–	–	–	–
R-7	–	–	20.7	55.5	–	–	21.2	55.8	–	–	–	–
N-CH_3	–	–	–	–	28.9	29.4	29.1	29.0	–	–	–	–

this compound had been prepared together with the isomer **8** (*figure 3*) and identified by ^{1}H-NMR chemical shift of the triazole H(5) (8.34 δ) which appears downfield of the corresponding triazole H(4) (8.23 δ for compound **8**); in addition its reduction gave the corresponding amino derivative **9b**, while reduction of isomer **8** provided the cyclized compound **10b**. Similarly compounds **3a,c,d** showed chemical shift values of the H(5) protons between 8.29 and 8.35 δ and were converted to the corresponding aminoderivatives **9a,c,d** by catalytic hydrogenation (*figure 3*). The structures **2a–d** resulted from the previous assignment, considering also the easy decarboxylation of the 5-ethoxycarbonyl-1,2,3-triazoles.

3. Biochemistry

The triazoloquinoxaline compounds **6a–d**, **7a–d**, **10a,b** and **11a,b** underwent binding assays either to benzodiazepine receptors or adenosine A_1 and A_{2A} receptors. Their ability to inhibit benzodiazepine receptor binding was measured by the concentration capable of displacing [^{3}H] Ro 15-1788 from bovine brain membranes.

The inhibition of the binding toward the adenosine receptors was measured by the capability of compounds **6a–d**, **7a–d**, **10a,b** and **11a,b** to displace the ^{3}H–N^6-cyclohexyladenosine (CHA) from A_1-adenosine receptors in sheep cortical membranes and

Table IV. Mass and IR data for compounds **6**, **7**, **10** and **11**.

	Mass [*m/z* (%)]	IR (cm^{-1})
6a	258(9), 186(53), 173(41), 157(28), 145(91), 130(27), 117(16), 103(35), 90(100), 77(8)	1720, 1660, 1240, 1190, 1090
6b[a]	220(32), 207(18), 191(8), 179(32), 164(14), 151(12), 137(16), 124(50)	1730, 1670, 1250, 1190, 1090
6c	272(9), 200(90), 187(20), 171(80), 159(80), 144(30), 131(22), 117(25), 104(79), 77(100)	1740, 1670, 1240, 1090
6d	288(7), 216(100), 203(10), 187(12), 175(26), 160(12), 147(32), 133(9), 120(40), 77(33)	1730, 1690, 1270, 1250, 1070
7a	272(10), 200(70), 187(33), 172(31), 159(100), 144(25), 131(18), 117(17), 102(32), 77(48)	1730, 1670, 1270, 1240, 1090
7b[a]	234(65), 221(28), 206(27), 193(69), 178(21), 165(21), 151(13), 138(19), 124(24), 100(33)	1730, 1680, 1270, 1150, 1090
7c	286(10), 214(100), 201(16), 186(38), 173(60), 157(30), 145(17), 131(11), 118(15), 77(28)	1710, 1670, 1250, 1210, 1090
7d	302(3), 230(100), 215(67), 202(14), 189(30), 187(44), 159(20), 130(15), 118(26), 77(24)	1730, 1690, 1270, 1250, 1070
10a	186(33), 158(32), 130(46), 103(100), 90(33), 76(44)	
10b	220(19), 192(30), 164(56), 137(100), 124(22), 110(23), 102(35), 76(39)	1690, 1250, 1090
11a[a]	202(1), 186(10), 158(13), 130(31), 103(100), 90(36), 76(83)	
11b[a]	220(20), 192(39), 164(68), 137(100), 124(20), 110(20), 102(44), 76(15)	1710, 1210, 1070

[a]The molecular ion was not detected even upon lowering the ionization potential.

^{3}H-2-[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS-21680) from A_{2A}-adenosine receptors in rat striatal membranes.

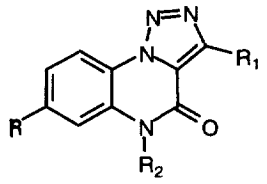
4. Results and discussion

The results in *table V* show that the triazoloquinoxaline derivatives have a high affinity toward the benzodiazepine receptors with values between 53 and 314 nM. The ethyl esters of the two series **6a–d** and **7a–d**, which are different for the presence of a hydrogen atom or a methyl group respectively on the N-5 atom, show a gradual change of receptorial affinity, depending upon the substituent in the 7 position of the tricyclic ring, according to the following sequence: Cl > OCH_3 > H > CH_3. In addition, considering that the NH function (Series **6**) can behave as a hydrogen bond donor while the NCH_3 function (Series **7**) can act as a possible acceptor, the comparison between the two series **6** and **7** shows some differences in affinity values which clearly involve the substituent on the phenyl ring; nevertheless this substituent affects the physico-chemical properties of the molecule rather than taking part in the receptorial binding.

Thus the chlorosubstituted compounds **6b** and **7b**, which are the more effective, present equivalent receptorial affinity (K_i = 53 nM and 54 nM respectively); the *N*-methyl derivatives, methoxy-substituted **7d** and unsubstituted **7a**, show a moderate increase of affinity compared to the corresponding unmethylated **6d** and **6a**; the less active compounds of the two series bearing a methyl substituent on the phenyl ring, present reversed affinity values (**7c**: K_i = 314 nM; **6c**: K_i = 242 nM). The acid derivatives **11a** and **11b** and the corresponding decarboxylated compounds **10a** and **10b** do not present any activity.

The GABA ratio values are not equivalent and show changes which do not agree with the affinity behaviour of the two series, **6** (N-5 unsubstituted) and **7** (N-5 methylsubstituted). In fact **6a** can be considered a moderate agonist (1.60) while **6b** (1.23), **6c**

Table V. Binding to benzodiazepine and to A_1 adenosine receptors.



Compound	R	R_1	R_2	Benzodiazepine K_i (nM)	GABA ratio	Adenosine A_1 K_i (nM)
6a	H	$COOC_2H_5$	H	228	1.60	816
6b	Cl	$COOC_2H_5$	H	53	1.23	79
6c	CH_3	$COOC_2H_5$	H	242	1.20	49.5
6d	OCH_3	$COOC_2H_5$	H	130	1.30	29
7a	H	$COOC_2H_5$	CH_3	182	1.68	–[a]
7b	Cl	$COOC_2H_5$	CH_3	54	1.60	–[a]
7c	CH_3	$COOC_2H_5$	CH_3	314	2.20	90
7d	OCH_3	$COOC_2H_5$	CH_3	101.5	0.99	389
10a	H	H	H	–[a]	–	–[a]
10b	Cl	H	H	–[a]	–	468
11a	H	COOH	H	–[a]	–	–[a]
11b	Cl	COOH	H	–[a]	–	–[a]

[a]The K_i values were not calculated because the inhibition percentages at 1 μM were too low.

(1.20) and **6d** (1.30) act as inverse agonists. On the contrary compounds **7c** (2.20), **7a** (1.68) and **7b** (1.60) can be considered agonists, and **7d** (0.99) as a weak antagonist.

In fact our structures **6** and **7** possess functional groups (**6**: one hydrogen bond donor and more acceptors; **7**: only hydrogen bond acceptors) arranged in such a way that they are able to interact with the receptorial sites hypothesized for an agonist, inverse agonist or antagonist of the benzodiazepine receptors [9]. Thus the structure in *figure 4* (compound **6b**, GABA ratio 1.23), bearing the NH donor group and as acceptor groups the ethoxycarbonyl function (because the acid **11b** and the decarboxylated compound **10b** are lacking activity), the amide CO in the 4 position and the N-1 and/or N-2 of the triazole ring, corresponds to the structure of inverse agonists/antagonists. It is worth noting that the ethoxycarbonyl function can form a three-point bond involving also the other acceptors (amide CO with H_1 site or the triazole N-2 with H_2 site). On the contrary the structure of compounds **7a–c** (GABA ratio 1.6–2.2), bearing the previous hydrogen bond acceptor groups and the methylated nitrogen in the 5 position as a possible further bond acceptor, corresponds to that of pure agonists.

The results in *table V* show that some triazoloquinoxaline derivatives herein reported have a high affinity (K_i values < 100 nM) toward the adenosine A_1 receptorial subtype too, with good specificity. In fact the binding assays to the adenosine A_{2A} receptors show that the more active compound **6d** presents only a 27% inhibition at 1000 nM contrary to the high adenosine A_1 receptor affinity (K_i = 29 nM); for the other compounds the inhibition percentages toward the A_{2A} receptors were much lower.

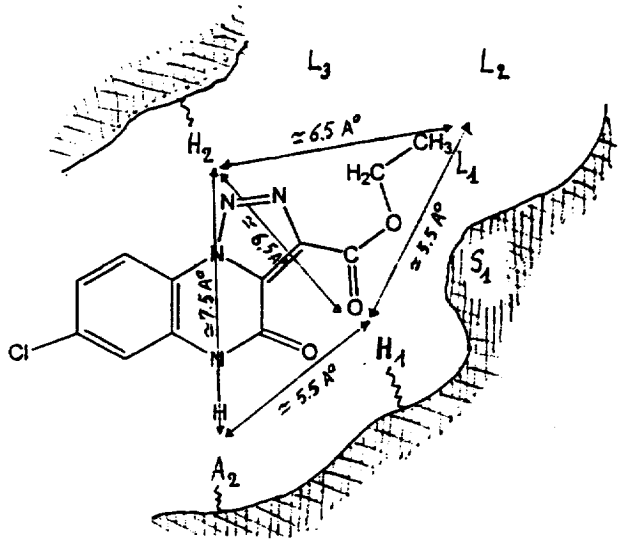


Figure 4. Interaction of compound **6b** with the benzodiazepine receptor; pharmacophoric structure [4].

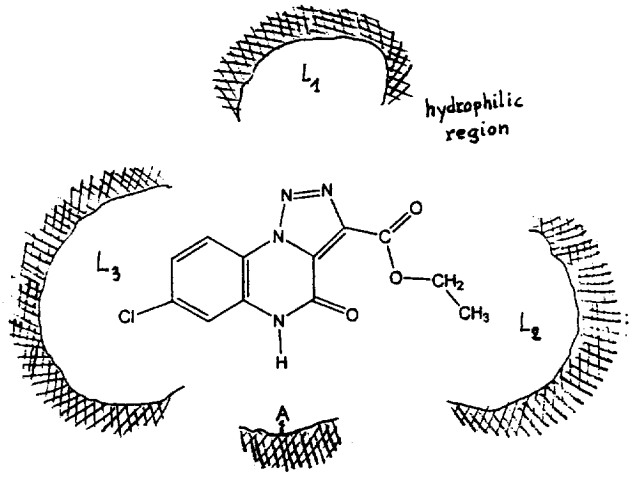


Figure 5. Interaction of compound **6b** with the adenosine A_1 receptor.

The more effective compounds belong to series **6**, bearing a hydrogen atom on the central nitrogen in the 5 position; their biological activity changes according to the phenyl substituent as follows: $OCH_3 > CH_3 > Cl > H$. Methylation of the N-5, a potential hydrogen bond donor, causes a 50% decrease of the affinity for the 7-methyl-substituted compound **7c** and a stronger decrease (~ 13-fold) for the 7-methoxy compound **7d**.

Hydrolysis of the ester function of **6a** and **6b** indicates that the acid group takes away activity (compounds **11a** and **11b**) while decarboxylation (compounds **10a** and **10b**) lowers the activity ~ 6-fold compared to the corresponding ester.

Figure 5 shows a probable interaction of the compound **6b** with the receptorial sites previously proposed for the adenosine A_1 receptors, obtained from studies of 1,2,3-triazolo[4,5-d]pyrimidines [10–14] and 1,2,3-triazolo[4,5-d]pyridazines [15–18]. This hypothesis also agrees with the actual receptorial models for adenosine agonists and antagonists [19].

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Kofler hot-stage and are uncorrected. IR spectra in nujol mulls were recorded on a Perkin-Elmer Model 1310 spectrometer. ^{1}H-NMR spectra were recorded with a Bruker AC 200 or a Varian Model CFT 20 spectrometer in δ units from TMS as an internal standard. Mass spectra were performed with a Hewlett Packard MS/System 5988. Elemental analyses (C, H, N) were within ±0.4% of the theoretical values and were performed on a Carlo Erba Elemental Analyzer Model 1106 apparatus. Column chromatographies were performed on Silica gel 60 (230–400 mesh). Petroleum ether corresponds to the fraction boiling at 40–60 °C.

5.2. 2-Nitrophenylazides

All the azides had been described in the literature and were prepared from the appropriate primary aromatic amine via diazonium salt and treatment with sodium azide: 2-nitrophenylazide [20], 4-chloro-2-nitrophenylazide [8], 4-methyl-2-nitrophenylazide [21] and 4-methoxy-2-nitrophenylazide [22].

5.2.1. Reaction between 2-nitrophenylazide and diethyl oxalacetate

A suspension of diethyl oxalacetate sodium salt (4.15 g, 19.7 mmol) in 70 mL of absolute EtOH was heated at 35–40 °C under stirring until complete solution. A solution of 2-nitrophenylazide (2.50 g, 15.2 mmol) in 60 mL of absolute EtOH was slowly dropped (~ 30 min), the temperature was rised to 55–58 °C and then the mixture was stirred for 7 h. After a night at room temperature, the reaction mixture was concentrated in vacuo (temperature < 60 °C) and the residue was treated with H_2O and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and evaporated to give a residue (2.13 g) consisting of a mixture of four compounds (TLC analysis, petroleum ether/AcOEt (2:1)). This mixture underwent flash-chromatography (silica gel column 14 x 4 cm). Elution with petroleum ether/AcOEt (2:1) provided 0.500 g (yield 10%) of benzoxadiazole N-oxide **4a** [20] followed by 0.180 g (yield 3.5%) of 2-nitroaniline **5a**. Elution with petroleum ether/AcOEt (1:1) provided 1.090 g (yield 21%) of the

triazole diester **1a** followed by 0.360 g (yield 7%) of the triazole monoester **3a**. Acidification of the aqueous alkaline layer caused precipitation of a crude semisolid material which was extracted with $CHCl_3$. Evaporation of the solvent gave a residue (2.80 g) from which, by washing with a mixture of petroleum ether/AcOEt (2:1), the 1-(2-nitrophenyl)-4-ethoxycarbonyl-5-carboxy-1H-1,2,3-triazole **2a** was isolated (2.00g, 39% yield).

1a: mp = 54–57 °C (EtOH); Ms *m/e* 335 (MH^+). Anal. $C_{14}H_{14}N_4O_6$ (C, H, N);

2a: mp = 131–134 °C (DMF-H_2O). Anal. $C_{12}H_{10}N_4O_6$ (C, H, N);

3a: mp = 77–78 °C (EtOH); Ms *m/e* 262 (M^+). ^{1}H-NMR ($CDCl_3$): 8.35 (s, 1H), 8.18–7.60 (m, 4H), 4.44 (q, 2H), 1.43 (t, 3H). Anal. $C_{11}H_{10}N_4O_4$ (C, H, N).

5.2.2. Reaction between 4-chloro-2-nitrophenylazide and diethyl oxalacetate

A suspension of diethyl oxalacetate sodium salt (5.88 g, 28.0 mmol) in 140 mL of absolute EtOH was heated at 35–40 °C under stirring until complete solution. A solution of 4-chloro-2-nitrophenylazide (5.54 g, 28.0 mmol) in 150 mL of absolute EtOH was slowly dropped (~ 40 min), the temperature was rised to 55 °C and then the mixture was stirred for 4 h. After a night at room temperature, the reaction mixture was worked up as described for the previous reaction. The residue obtained from the organic layer (6.50 g) underwent flash-chromatography (silica gel column 14 x 6 cm). Elution with petroleum ether/AcOEt (4:1) provided the benzoxadiazole N-oxide **4b** [8] (1.050 g, yield 10%); elution with petroleum ether/AcOEt (3:1) provided the chloronitroaniline **5b** (0.600 g, yield 6%); elution with petroleum ether/AcOEt (2:1) gave 2.50 g (yield 24%) of the triazole diester **1b**, followed by 0.833 g (yield 8%) of the triazole monoester **3b**. Acidification of the aqueous alkaline layer provided a crude precipitate (3.80 g) from which, by washing with a mixture of petroleum ether/AcOEt (2:1), the acid triazole derivative **2b** (3.10 g, yield 30%) was isolated.

1b: mp = 80–81 °C (EtOH); Ms *m/e* 369 (M^+). Anal $C_{14}H_{13}N_4O_6Cl$ (C, H, N);

2b: mp = 135–138 °C (DMF-H_2O). Anal $C_{12}H_9N_4O_6Cl$ (C, H, N);

3b: mp = 148–149 °C (EtOH); Ms *m/e* 297 (M^+). ^{1}H-NMR ($CDCl_3$): 8.34 (s, 1H), 8.12–7.51 (m, 3H), 4.44 (q, 2H), 1.43 (t, 3H). Anal. $C_{11}H_9N_4O_4Cl$ (C, H, N).

5.2.3. Reaction between 4-methyl-2-nitrophenylazide and diethyl oxalacetate

A suspension of diethyl oxalacetate sodium salt (1.60 g, 7.60 mmol) in 30 mL of absolute EtOH was heated at 35–40 °C under stirring until complete solution. A solution of 4-methyl-2-nitrophenylazide (1.10 g, 6.20 mmol) in 70 mL of absolute EtOH was slowly dropped (~ 40 min), the temperature was rised to 50 °C and then the mixture was stirred for 22 h. The reaction mixture was worked up as described for the previous reaction and flash-chromatography (silica gel column 14 x 4 cm) of the residue obtained from the organic layer (1.15 g) under elution gradient (petroleum ether/AcOEt = 4:1; 3:1; 2:1) provided: benzoxadiazole N-oxide **4c** [21] (0.080 g, yield 3.7%); methylnitroaniline **5c** (0.100 g, yield 4.7%); triazole diester **1c** (0.680 g, yield 32%) and triazole monoester **3c** (0.194 g, yield 9%). From the aqueous alkaline layer by acidification, the acid triazole derivative **2c** (0.730 g, yield 34%) was isolated.

1c: mp = 92–93 °C (EtOH); Ms *m/e* 348 (M^+). Anal. $C_{15}H_{16}N_4O_6$ (C, H, N);

2c: mp = 130–133 °C (DMF-H_2O). Anal. $C_{13}H_{12}N_4O_6$ (C, H, N);

3c: mp = 67–70 °C (EtOH); Ms *m/e* 276 (M^+). ^{1}H-NMR ($CDCl_3$): 8.30 (s, 1H), 7.91–7.40 (m, 3H), 4.44 (q, 2H), 2.56 (s, 3H), 1.43 (t, 3H). Anal. $C_{12}H_{12}N_4O_4$ (C, H, N).

5.2.4. Reaction between 4-methoxy-2-nitrophenylazide and diethyl oxalacetate

A suspension of diethyl oxalacetate sodium salt (3.00 g, 14.3 mmol) in 30 mL of absolute EtOH was heated at 35–40 °C under stirring until complete solution. A solution of 4-methoxy-2-nitrophenylazide (2.40 g, 12.4 mmol) in 140 mL of absolute EtOH was slowly dropped (~ 40 min), the temperature was rised to 55 °C and then the mixture was stirred for 24 h. The reaction mixture was worked up as described for the previous reaction and flash-chromatography (silica gel column 14 x 4 cm) of the residue obtained from the organic layer (2.50 g) under elution gradient (petroleum ether/AcOEt = 4:1; 3:1; 2:1) provided: benzoxadiazole N-oxide **4d** [22] (0.310 g, yield 7%); methoxynitroaniline **5d** (0.221 g, yield 5%); triazole diester **1d** (0.880 g, yield 20%) and triazole monoester **3d** (0.922 g, yield 21%). From the aqueous alkaline layer by acidification, the acid triazole derivative **2d** (1.5 g, yield 33%) was isolated.

1d: mp = 77–80 °C (EtOH); Ms *m/e* 364 (M^+). Anal. $C_{15}H_{16}N_4O_7$ (C, H, N);

2d: mp = 143–146 °C (DMF-H_2O). Anal. $C_{13}H_{12}N_4O_7$ (C, H, N);

3d: mp = 93–95 °C (EtOH); Ms *m/e* 292 (M^+). ^{1}H-NMR ($CDCl_3$): 8.29 (s, 1H), 7.61–7.20 (m, 3H), 4.44 (q, 2H), 3.96 (s, 3H), 1.43 (t, 3H). Anal. $C_{12}H_{12}N_4O_5$ (C, H, N).

5.3. 3-Ethoxycarbonyl-1,2,3-triazolo[1,5-a]quinoxalin-4-one ***6a****, 3-ethoxycarbonyl-7-methyl-1,2,3-triazolo[1,5-a]quinoxalin-4-one* ***6c*** *and 3-ethoxycarbonyl-7-methoxy-1,2,3-triazolo[1,5-a]quinoxalin-4-one* ***6d***

To a solution of the appropriate nitrotriazole derivative **1a**, **1c** or **1d** (3.0 mmol) in ~ 250 mL of EtOH was added 5% Pd/C (~ 0.100 g) and the mixture hydrogenated at room temperature and pressure. The suspension was heated under reflux and the catalyst was filtered off and washed with boiling EtOH. The combined filtrates were evaporated in vacuo to give the title compounds (*table I*).

5.3.1. 3-Ethoxycarbonyl-7-chloro-1,2,3-triazolo[1,5-a]quinoxalin-4-one ***6b***

To a solution of 1-(4-chloro-2-nitrophenyl)-4,5-di-ethoxycarbonyl-1H-1,2,3-triazole **1b** (2.30 g, 6.24 mmol) in 140 mL of 60% EtOH was added aqueous 5% $FeCl_3$ (1.2 mL) and iron powder (1.20 g) and the mixture was refluxed for 4 h. The boiling reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was treated with H_2O and the obtained suspension was alkalinized with 32% ammonium hydroxide solution and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and evaporated to give **6b** (*table I*).

5.4. 3-Ethoxycarbonyl-5-methyl-1,2,3-triazolo[1,5-a]quinoxalin-4-one ***7a****, 3-ethoxycarbonyl-5-methyl-7-chloro-1,2,3-triazolo[1,5-a]quinoxalin-4-one* ***7b****, 3-ethoxycarbonyl-5,7-dimethyl-1,2,3-triazolo[1,5-a]quinoxalin-4-one* ***7c*** *and 3-ethoxycarbonyl-5-methyl-7-methoxy-1,2,3-triazolo[1,5-a]quinoxalin-4-one* ***7d***

A mixture of 2.0 mmol of the appropriate triazoloquinoxaline **6a–d**, 1.2 mL (12.6 mmol) of Me_2SO_4 and 0.750 g (5.1 mmol) of anhydrous K_2CO_3 in 40 mL of anhydrous

acetone (for **6a** and **6b**) or in 12 mL of anhydrous DMF (for **6c** and **6d**) was refluxed or heated at 120 °C respectively, for 4 h. The reaction mixture was concentrated in vacuo, the residue was treated with H_2O and the separated solid, being the title compounds, was collected by filtration (*table I*).

5.5. Reaction between 4-chloro-2-nitrophenylazide and ethyl propiolate

To a solution of 2-nitro-4-chlorophenylazide (1.05 g, 5.29 mmol) in 2 mL of anhydrous DMF was added 2 mL (19.7 mmol) of ethyl propiolate and the mixture heated at 45 °C for 48 h. The reaction mixture was concentrated in vacuo, then treated with H_2O and extracted with $CHCl_3$. The organic layer, after washing with H_2O, was dried and evaporated to give 1.25 g of a crude solid consisting of the mixture of the two triazole isomers. This residue was fractionated by flash chromatography through silica gel column (12 x 4 cm) eluting with petroleum ether/AcOEt (2:1). Firstly the isomer 1-(2-nitro-4-chlorophenyl)-5-ethoxycarbonyl-1H-1,2,3-triazole **8** (0.220 g, yield 14%) was eluted, followed by the 1-(2-nitro-4-chlorophenyl)-4-ethoxycarbonyl-1H-1,2,3-triazole **3b** (0.690 g, yield 44%).

8: mp = 122–123 °C (EtOH); Ms *m/e* 297 (M^+). ^{1}H-NMR ($CDCl_3$): 8.23 (s, 2H), 7.63–7.43 (m, 2H), 4.25 (q, 2H), 1.29 (t, 3H). Anal. $C_{11}H_{10}N_4O_4Cl$ (C, H, N).

5.6. 1-(2-Amino-4-chlorophenyl)-4-ethoxycarbonyl-1H-1,2,3-triazole

To a solution of 1-(2-nitro-4-chlorophenyl)-4-ethoxycarbonyl-1H-1,2,3-triazole **3b** (0.450 g, 1.51 mmol) in 40 mL of 60% EtOH was added aqueous 5% $FeCl_3$ (0.2 mL) and iron powder (0.230 g) and the mixture was refluxed for 3 h. The reaction mixture was worked up as described for the preparation of **6b**. Evaporation of the chloroformic layer provided 0.364 g (yield 90%) of **9b**: mp = 109–110 °C (EtOH); Ms *m/e* 266 (M^+). Anal. $C_{11}H_{11}N_4O_2Cl$ (C, H, N).

*5.7. 1-(2-Aminophenyl)-4-ethoxycarbonyl-1H-1,2,3-triazole **9a**, 1-(2-amino-4-methylphenyl)-4-ethoxycarbonyl-1H-1,2,3-triazole **9c** and 1-(2-amino-4-methoxyphenyl)-4-ethoxycarbonyl-1H-1,2,3-triazole **9d***

To a solution of the appropriate nitrotriazole derivative **3a**, **3c** or **3d** (1.0 mmol) in 80 mL of EtOH was added 5% Pd/C (~ 0.030 g) and the mixture hydrogenated at room temperature and pressure. The suspension was heated under reflux and the catalyst was filtered off and washed with boiling EtOH. The combined filtrates were evaporated in vacuo to give the title compounds.

9a: yield 96%; mp = 114–115 °C (EtOH); Ms *m/e* 232 (M^+). Anal. $C_{11}H_{12}N_4O_2$ (C, H, N);

9c: yield 95%; mp = 101–103 °C (EtOH); Ms *m/e* 246 (M^+). Anal. $C_{12}H_{14}N_4O_2$ (C, H, N);

9d: yield 95%; mp = 120–122 °C (EtOH); Ms *m/e* 262 (M^+). Anal. $C_{12}H_{14}N_4O_3$ (C, H, N).

*5.8. 7-Chloro-1,2,3-triazolo[1,5-a]quinoxalin-4-one **10b***

Method A: To a solution of 1-(2-nitro-4-chlorophenyl)-5-ethoxycarbonyl-1H-1,2,3-triazole **8** (0.340 g, 1.15 mmol) in 30 mL of 60% EtOH was added aqueous 5% $FeCl_3$ (0.2 mL) and iron powder (0.200 g) and the mixture was refluxed for 6 h. The reaction mixture was worked up as described for the preparation of **6b** to obtain the title compound (*table I*).

Method B: A mixture of **11b** (0.050 g, 0.18 mmol) in Dowtherm (2 mL) was refluxed for 2.5 h; after cooling the reaction mixture was diluted with petroleum ether to precipitate the title compound which was collected by filtration: 0.036 g (yield 90%).

*5.9. 3-Carboxy-1,2,3-triazolo[1,5-a]quinoxalin-4-one **11a** and 3-carboxy-7-chloro-1,2,3-triazolo[1,5-a]quinoxalin-4-one **11b***

Method A: To a solution of 0.77 mmol of **6a** or **6b** in 10 mL of DMSO, 3.5 mL (3.12 mmol) of 5% ethanolic KOH was added and the mixture stirred at room temperature for 24 h. The precipitated potassium salt was dissolved by adding H_2O and acidification of the solution with 36% HCl gave the title compounds which were collected by filtration (*table I*).

Method B: To a hot solution of 1.6 mmol of **6a** or **6b** in 40 mL of EtOH, solid Na_2CO_3 (1.0 g, 9.43 mmol) was added and the mixture refluxed for 24 h. The hot solution was filtered and the solid collected was treated with H_2O and acidified by 36% HCl to give the title compounds as a white solid in 80% yield.

*5.10. 1,2,3-triazolo[1,5-a]quinoxalin-4-one **10a***

A mixture of **11a** (0.080 g, 0.35 mmol) in Dowtherm (4 mL) was refluxed for 2.5 h. The reaction mixture was worked up as described for the preparation of **10b** to obtain the title compound (*table I*).

5.11. Biochemistry

5.11.1. Benzodiazepine receptor binding

Tritiated [^{3}H]Ro15-1788 was obtained from Du Pont de Nemours, New England Nuclear Division (Dreieichenhaim, Germany) and had a specific activity of 83.6 Ci/mmol and a radiochemical purity > 99%. All other chemicals were of reagent grade and obtained from commercial suppliers. Bovine cerebral cortex was rapidly isolated and homogenized in 10 volumes of ice cold 0.32 M sucrose containing protease inhibitors [23]. The homogenate was centrifuged at 1000 *g* for 10 min at 4 °C. The resulting pellet was discarded and the supernatant was recentrifuged at 50000 *g* for 30 min at 4 °C. Then the pellet was osmotically shocked by suspension in 10 vol of 50 mM Tris Citrate buffer at pH 7.4 containing protease inhibitors and recentrifuged at 50000 *g* for 30 min at 4 °C. The resulting membranes were frozen and washed using a procedure previously described for removing endogenous GABA from the rat cerebral cortex [24]. Finally, the pellet was suspended in 10 vol of 50 mM Tris Citrate buffer at pH 7.4 and used in the binding assay. Protein concentration was assayed by the method of Lowry et al. [25].

Routine binding assays were made by incubating 0.4 mg protein of crude bovine brain membrane suspensions at 0 °C for 90 min with [^{3}H]Ro15-1788 (0.2 nM) in a total volume of 0.5 mL of Tris Citrate buffer. After incubation, the samples were diluted at 0 °C with 5 mL of the assay buffer and were immediately filtered under reduced pressure through glass fiber filter disks (Whatman GF/B). The filters were washed twice with 5 mL of the buffer, dried and added to 8 mL of Ready Protein Beckman scintillation cocktail and radioactivity was counted in an LS 1800 scintillation counter. Nonspecific binding was determined by the radioactivity bound in the presence of 10 μM non-radioactive diazepam in parallel assays. Water-insoluble derivatives were dissolved in EtOH or DMSO and added to the assay mixture. Blank experiments were carried out to determine the effect of EtOH (2%) or DMSO

(1%) on binding. The inhibition of specific binding was determined in the presence of a single concentration (10 μM) of the potential displacing agent. For the most active compounds the concentration inhibiting specific binding by 50% (IC_{50}) was calculated from the displacement curves by log–probit analysis with four to six concentrations of the displacers each performed in triplicate. The dissociation constant (K_i) was derived according to the equation of Cheng and Prusoff [26]; the ligand affinity (K_d) of [^{3}H]Ro15-1788 was 0.64 nM.

5.11.2. A_1 adenosine receptor binding

Bovine cerebral cortex was homogenized in icecold 0.32 M sucrose containing protease inhibitors, as previously described [27]. The homogenate was centrifuged at 1000 *g* for 10 min at 4 °C and the supernatant again centrifuged at 48000 *g* for 15 min at 4 °C. The final pellet was dispersed in 10 volumes of fresh buffer, incubated with adenosine deaminase (2 units/mL) to remove endogenous adenosine at 37 °C for 60 min, and then recentrifuged at 48000 *g* for 15 min at 4 °C. The pellet was suspended in buffer and used in the binding assay.

The [^{3}H]CHA binding assay was performed in triplicate by incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) at 25 °C for 45 min in 0.5 mL of Tris-HCl, pH 7.7, containing 2mM $MgCl_2$, with approximately 1.2 nM [^{3}H]CHA. Nonspecific binding was defined in the presence of 50 μM *R*-PIA. The assay was completed by filtration through Whatman GF/C glass microfibre filters under suction and washing twice with 5 mL of ice-cold buffer.

5.11.3. A_{2A} adenosine receptor binding

Bovine striatum was homogenized in 20 volumes of icecold 50 mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$, and protease inhibitors. The membrane homogenate was centrifuged at 48000 *g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 units/mL of adenosine deaminase and incubated at 37 °C for 30 min. The membrane homogenate was centrifuged, and the final pellet was frozen at –80 °C. Routine assays were performed in triplicate by incubating an aliquot of striatal membranes (0.2–0.3 mg of protein) in 50 mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$ with approximately 5 nM [^{3}H] CGS 21680 in a final volume of 0.5 mL. Incubation was carried out at 25 °C for 90 min. Nonspecific binding was defined in the presence of 50 μM CGS 21680. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of icecold buffer and placed in scintillation vials. The radioactivity was counted in a 4 mL Beckman Ready-Protein scintillation cocktail in a scintillation counter. The compounds were dissolved in DMSO and added to the assay mixture to make a final volume of 0.5 mL. Blank experiments were carried out to determine the effect of the solvent (2%) on the binding. The concentration of the tested compounds to produce 50% inhibition of specific [^{3}H]CHA or [^{3}H]CGS 21680 binding (IC_{50}) was determined from semilog plots of data from experiments of binding inhibition. The K_i values were calculated from the IC_{50} values using the equation $IC_{50}/(L/K_d)$ [26]. For [^{3}H]CHA, K_d = 10.5 nM and L = 1.2 nM; for [^{3}H]CGS 21680, K_d = 1 nM and L = 5 nM. Protein estimation was based on the method reported in [25], using bovine serum albumin as standard.

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