Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 23, pp. 2735-2740, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00409-9

Synthesis and SAR of 2H-1,2,4-Benzothiadiazine-1,1-dioxide-3-carboxylic acid Derivatives as Novel Potent Glycine Antagonists of the NMDA Receptor-Channel Complex

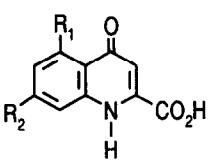
Patrick Jimonet,§ François Audiau, Jean-Claude Aloup, Michel Barreau, Jean-Charles Blanchard, Andrees Bohme, Alain Boireau, Michel Chevé, Dominique Damour, Adam Doble, Jacques Lavayre, Gilles Dutruc-Rosset, John C. R. Randle, Jean Rataud, Jean-Marie Stutzmann and Serge Mignani *

RHONE-POULENC RORER S.A. Centre de Recherches de Vitry-Alfortville, 13 Quai Jules Guesde-BP 14, 94403 Vitry-sur-Seine Cedex, France

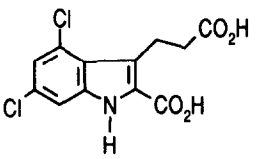
Abstract: *A new series of 2H-1,2,4-benzothiadiazine-1,1-dioxide-3-carboxylic acids was synthesized. Most compounds demonstrated excellent binding affinities at the glycine binding site of the NMDA receptor-channel complex. A member of this series,* ***RPR 104632*** *displaces strychnine-insensitive [^{3}H]-DCKA binding with high potency (IC_{50} = 8 nM) and stereoselectivity: the levorotatory isomer* ***(-)-8k*** *(IC_{50} = 4 nM) is about 500-times more potent than the dextrorotatory isomer* ***(+)-8k*** *(IC_{50} = 1880 nM). In <u>in vitro</u> and <u>in vivo</u> models,* ***RPR 104632*** *is a potent antagonist of central NMDA receptor activation.*

L-glutamic acid is the major endogeneous excitatory amino acid (EAA) neurotransmitter in the mammalian central nervous system. There is considerable evidence that the overstimulation of excitatory amino acid receptors, notably the NMDA (N-methyl-D-aspartic acid) and AMPA [2-amino-3-(3-hydroxy-5-methylisoxazole-4-yl)propionic acid] receptor sub-types, may be associated with various acute and chronic neurodegenerative disorders.[1]

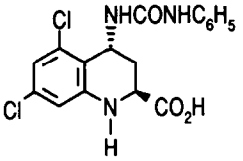
Johnson and Ascher (1987) demonstrated that glycine acts as a coagonist at a specific site on the NMDA receptor[2] to amplify the agonist action of glutamic acid. This mechanism is independent of its known action on the strychnine-sensitive glycine receptor. These findings have led to an intensive search for NMDA antagonists that inhibit this action of glycine. Several compounds belonging to various chemical families have been shown to be effective glycine-site NMDA antagonists.[3]



R1 = R2 = H **1**
R1 = R2 = Cl **DCKA**



2



L-689,560

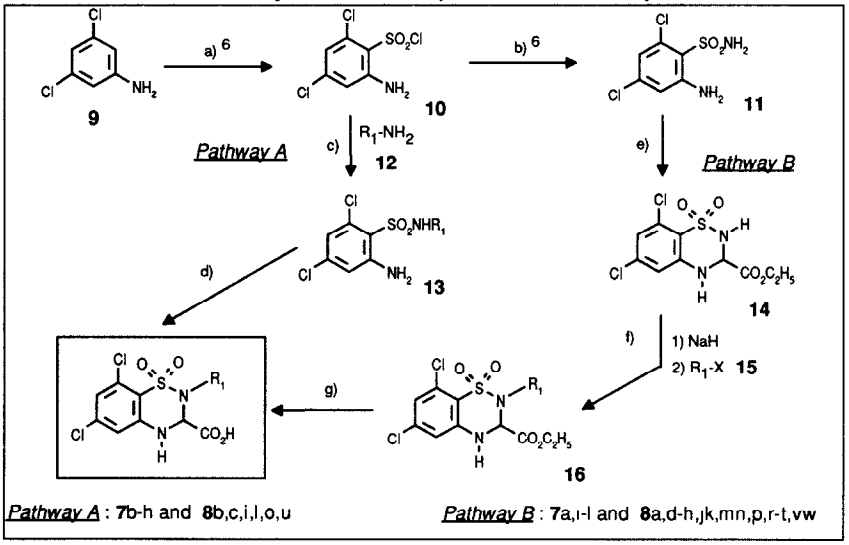
Dedicaced to Prof. H. G. VIEHE on the occasion of his 65th birthday

Table 1: *In Vitro* Activities of 2, 7a-m , 8a-w, DCKA, and L-689,560

7	R_1	IC_{50} [a]	8[c]	R_4	IC_{50} [a]	8[c]	R_4	IC_{50} [a]
7a	$-CH_3$	*400*	**8a**	2-CH_3	***80***	**8m**	3-CN	*17*
7b	$-C_6H_5$	*2600*	**8b**	2-OCH_3	***21***	**8n**	3-NO_2	*12*
7c	$-CH_2-C_6H_5$	*48*	**8c**	2-CF_3	***100***	**8o**	3-OCF_3	*20*
7d	$-(CH_2)_2-C_6H_5$	*90*	**8d**	2-F	***26***	**8p**	3-OC_6H_5	*9*
7e	$-(CH_2)_3-C_6H_5$	*33*	**8e**	2-Cl	***48***	**8q**	3-NH_2	*29*
7f	$-(CH_2)_4-C_6H_5$	*77*	**8f**	3-CH_3	***14***	**8r**	3-C_6H_5	*20*
7g	$-(CH_2)_5-C_6H_5$	*36*	**8g**	3-OCH_3	***12***	**8s**	4-CH_3	*45*
7h	$-CH_2-CO_2H$	*9500*	**8h**	3-CF_3	***18***	**8t**	4-OCH_3	*40*
7i	$-(CH_2)_2-N(CH_3)_2$	*3600*	**8i**	3-F	***13***	**8u**	4-CF_3	*92*
7j	$-CH_2-CO-C_6H_5$	*22*	**8j**	3-Cl	***11***	**8v**	4-F	*44*
7k	$-(CH_2)_2OC_6H_5$	*41*	**8k**[b]	3-Br	***8***	**8w**	4-Cl	*20*
7l	$-(CH_2)_2SC_6H_5$	*38*	**(-)-8k**	3-Br	***4***	**DCKA**		*46*
7m	$-(CH_2)_2SO_2C_6H_5$	*70*	**(+)-8k**	3-Br	***1880***	**2**		*50*
			8l	3-I	***8***	**L-689,560**		*26*

[a]: IC_{50} values (nM) are the mean of the last 3 determinations each with 6 concentrations of test compounds triplate. [b]: **RPR 104632** [c]: $R_1 = CH_2(R_4\text{-Ph})$

Scheme 1: Synthesis of Compounds 7a-l and 8 a-p, r-w.

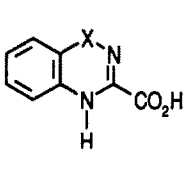


Reagents and reaction conditions: a) $ClSO_3H$, 105°C, 24 h, 84% crude **b)** liq. NH_3, 1.5 h, 73% **c)** R_1-NH_2, THF, $(C_2H_5)_3N$, rt, 10-24h **d)** OHC-CO_2H . H_2O, C_2H_5OH, H_2SO_4, reflux, 3 h then LiOH, THF-water mixture, rt, 12 h for compounds **7b** and **8u** or $(CH_3)_3SiOK$, CH_2Cl_2, rt, 24 h for compound **7c** or then 0.5N NaOH, rt, 12 h for compounds **7d** and **8bl**; OHC-CO_2H . H_2O, 1,4-dioxane-water mixture, reflux, 2-8 h for compounds **7e-h** and **8c,l,o e)** OHC-CO_2H . H_2O, C_2H_5OH, H_2SO_4, reflux, 1 h **f)** NaH, THF, rt then R_1-X (X = Cl or Br) in THF, reflux 2-5 h **g)** $(CH_3)_3SiOK$, CH_2Cl_2, rt, 1-24 h for compounds **7a,k**; LiOH, THF-water mixture, rt, 24 h for compounds **8a,d-h,j,s,t,v,w**; 0.5N NaOH, rt, 24 h for compounds **7i,j,l** and **8k,m,n,p,r.**

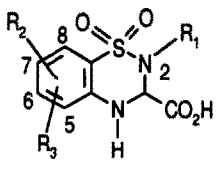
A number of these agents, such as kynurenic acid derivatives (*e.g.* 5,7-dichlorokynurenic acid, **DCKA**), 2-carboxy-indole-3-propionic acid derivatives (*e.g.* **2**) and 5,7-dichloro-4-ureido-2-carboxytetrahydroquinoline derivatives (*e.g.* **L-689,560**) have been reported [3] to be antagonists of the NMDA receptor-channel complex. In our assays,[4] these compounds display IC_{50} values for the glycine modulatory site of 46, 50 and 26 nM, respectively (Table 1).

This paper reports the synthesis and SAR of a new class of potent and selective glycine-NMDA antagonists **7a-m** and **8a-w** (Table 1 and Scheme 1). One of them, compound **8k** (**RPR 104632**) displays excellent affinities for the glycine binding site, and possesses potent antagonist properties in functional models. The biological activity is restricted to the levorotatory isomer **(-)-8k**.

We initially performed the synthesis of the quinazoline-2-carboxylic acid derivative **4**, which possesses the general structure of kynurenic acid **1**, with an additional nitrogen atom in the 3-position. The affinity for the glycine site decreased dramatically with respect to compound **1** (IC_{50} > 100 µM *vs.* 16 µM). Next, we replaced the carboxamido group of **4** by the corresponding sulfonamido moiety to give the benzothiadiazine **5**. Unexpectedly, this compound displayed a 10 µM affinity for the glycine site, close to that of kynurenic acid **1**. Starting from this interesting result, we synthesized 3,4-dihydro derivatives of these 2H-1,2,4-benzothiadiazine-1,1-dioxide-3-carboxylic acids with the general formula **6**. The parent compound **6a** (R_1,R_2,R_3=H) attained a similar affinity for the glycine binding site with an IC_{50} value of 8 µM.



4 X=CO
5 X=SO_2



6

It is well known that in the kynurenic acid, tetrahydroquinoline acid or 2-carboxy-indole acid series, affinity is greatly enhanced by appropriate introduction of chlorine atom(s) to the phenyl ring in position(s) 5, and 7, 5 and 7 or 4 and 6, respectively.[3] We observed the same effect in the 3,4-dihydro-1,2,4-benzothiadiazine series. Thus, the compounds **6a-h** [5] displayed affinities for the glycine site with the following rank order of binding: **6b** (R_1 = H; R_2,R_3 = 6,8-Cl; 0.073µM) > **6c** (R_2 = 6-Cl; R_1,R_3 = H; 0.18µM) > **6d** (R_1 = H; R_2,R_3 = 6,8-CH_3; 0.32µM) > **6e** (R_2 = 6-CH_3; R_1,R_3 = H; 0.41µM) > **6f** (R_2 = 8-I; R_1,R_3 = H; 0.79µM) > **6g** (R_1 = H; R_2,R_3 = 6,7-Cl; 1.9µM) > **6a** (R_1,R_2,R_3 = H; 8.5µM) > **6h** (R_1 = H; R_2,R_3 = 7,8-Cl; 27µM).

Starting from these results, we prepared the compounds **7a-m**[5] and the benzyl derivative analogs **8a-w** [5], varying the substituents in position 2 (Table 1).

Synthesis: *(Scheme 1)* Compounds **7a-l** and **8a-p,r-w** were synthesized by two approaches. The first approach (**pathway A**) was the reaction of the aminophenylsulphonyl chloride **10** [6] with the amine **12** in moderate to high yields (14 - 90%) to give sulphonamides **13**. The desired compounds **7b-h** and **8b,c,i,l,o,u** were obtained in

moderate to high yields (17 - 87%) from the corresponding sulphonamides **13**, by reaction with glyoxylic acid. The second approach (**pathway B**) required the addition of glyoxylic acid in acidic medium in the presence of ethanol to the sulphonamide **11** [6] to provide **14** in 86% yield. Then, the alkylation of the anion formed from **14** with the alkyl halides **15** afforded the corresponding ethyl-2-alkylbenzothiadiazinecarboxylate derivatives **16** in moderate to good yields (22 -61.5%). Aqueous basic hydrolysis of **16** gave **7a,l-l** and **8a,d-h,jk,mn,p,r-t,vw** in 25 - 94% yield.

The compound **7m** was synthesized by oxidation of **7l** (mCPBA, CH_2Cl_2, rt, 0.5 h) in 60 % yield whereas the compound **8q** was obtained by reduction of the corresponding nitro derivative **16** ($R_4 = NO_2$) under hydrogen pressure (1 bar, 5% Pd/C, ethanol, rt, 12 h) followed by an aqueous basic hydrolysis (0.5N NaOH) in 56% overall yield.

All new compounds have been caracterized by ^{1}H-NMR, IR and Mass Spectroscopy, and have given satisfactory combustion analyses (C, H, N, O, S). [7]

Biological Activity:

The IC_{50} values [4] for compounds **2**, **7a-m**, **8a-w**, **DCKA**, and **L-689,560** are shown in Table 1. Among the compounds prepared, the 3-bromobenzyl-derivative **8k** most potently displaced [^{3}H]-DCKA binding (IC_{50} = 8 nM). On the basis of the binding data, the following structure-activity relationships were observed:

❶ Introduction of an alkyl group in the 2-position: a methyl group reduced the binding affinity (**7a** *vs.* **6b**) while compound **7b** bearing a phenyl ring is much less active (IC_{50} = 2.6 µM) ❷ Introduction of phenylalkyl chains retained potency whatever the chain length (**7c-g** *vs* **6b**) ❸ Introduction of an alkyl chain bearing either a carboxylic acid or an amino function dramatically reduced potency (**7h** and **7i** *vs.* **6b**). ❹ Introduction of either an oxygen, sulfur atom or a sulfoxide moiety onto the phenylalkyl chain of compound **7d** had little effect on the affinity for the glycine site (**7k-m** *vs.* **6b**).

These results suggest the presence of a large bulk tolerance in the vicinity of the 2-substituent and a variety of groups are tolerated without loss of affinity. The excellent binding affinities of phenylalkyl-substituted benzothiazoline **7c** (IC_{50} = 48 nM) led us to choose the benzyl group for framework optimization. Consequently, a series of 2-benzylsubstituted compounds **8a-w** were prepared (Table 1). Activity is optimal when there is a substituent in the 3-position of the phenyl ring compared to substitution in the 2- or 4-positions (**8f-j** *vs.* **8a-e** or **8s-w**).

Derivatives bearing various 3-substituents such as electron-withdrawing groups (*e.g.* -CN, $-NO_2$), electron-donating groups (*e.g.* $-OCH_3$, $-OC_6H_5$, $-NH_2$) or halogen atoms are 2 to 5-fold more active than the corresponding parent compound **7c**. These results suggest that the increase in affinity of 3-substituted-benzyl derivatives relative to **7c** may be a consequence of enhanced hydrogen bonding ability of these polar groups.

As shown in Table 1, **RPR 104632** displays very high affinity for the [^{3}H]-DCKA binding site (IC_{50} = 8 nM), six-and three-fold more potent than **DCKA** and **L-689,560** respectively.

Given the high glycine-binding site affinity of racemic **RPR 104632**, we went on to examine both enantiomers **(+)-8k** and **(-)-8k** of this compound. The two enantiomers were prepared in optically pure form by HPLC using a column packed with a chiral stationary phase (Pirkle-type phase Chyrosine A from SEDERE-France). Enantiomeric homogeneity of both enantiomers (>99%) was evaluated by HPLC using the same chiral phase (Table 2).

Table 2: Chromatographic separation of enantiomers (+)-8k and (-)-8k

	retention time	weight	yield	$[\alpha]_D^{20}$ (c = 0.5 ; MeOH)
(+)-8k	24 mn	0.335 g	92%	+ 32.6 ± 1°
(-)-8k	30 mn	0.300 g	82%	- 30.7 ± 1.2°

As shown in Table 1, the levorotatory isomer **(-)-8k** displayed about a 500-fold greater potency at the glycine site than the dextrorotatory **(+)-8k** (IC_{50} = 4 *vs.* 1880 nM, respectively).

RPR 104632 showed potent and selective antagonist activity in several functional models.Thus, **RPR 104632** antagonized concentration-dependently (IC_{50} = 890 nM) the rise in cGMP levels induced by NMDA (80µM) stimulation [8] in immature rat cerebellar cortex, an effect that was reversed by addition of 1 mM glycine. Intravenous administration of 1-30 mg/kg **RPR 104632** reduced microiontophoretic responses of thalamic neurons to NMDA .[9] The maximal decrease reached 70 % inhibition and lasted for 60 min.*In vivo*, **RPR 104632**, injected i.c.v., dose-dependently protected DBA/2 mice from audiogenic seizures [10] with an ED_{50} of 2.5 µg/mouse. However, **RPR 104632** was inactive when injected systemically at 100 mg/kg i.p.

RPR 104632 proved to be highly selective for the glycine binding site. Considerable crossover of activity between the glycine-site and the AMPA receptor has been observed for previously-described series of antagonists. **RPR 104632** displaced [^{3}H]AMPA binding with an IC_{50} of 38 µM and is thus ~5000-fold selective for the glycine-site over the AMPA receptor. No displacement of radioligands for α_1-, α_2- or β-adrenergic, muscarinic, D_1- or D_2-dopamine, 5-HT_{2A}-serotonin or Ca^{2+} channel receptors was observed at concentrations of 10 µM.

In conclusion, **RPR 104632** is representative of a novel chemical series and is easily prepared in a five-step synthesis from commercially available reagents. In *in vitro* and *in vivo* models , **RPR 104632** is one of the most potent glycine site antagonist reported to date [11] in measures of stimulation of central NMDA receptors. Further pharmacological studies to evaluate the efficacy of **RPR 104632** and its enantiomer **(-)-8k** as neuroprotective agents are in progress and will be reported elsewhere.

Acknowledgements. We wish to thank B. Martin, P. Ganil, V. Mary for technical assistance, and M. Vuilhorgne and coworkers for analytical assistance. This work has been done as part of the Bio Avenir programme supported by Rhône-Poulenc with the participation of the French Ministry of Research and the French Ministry of Industry.

References and Notes.

§ To whom correspondence should be addressed.

1. For reviews, see: Choi, D. W. *Neuron*, **1988**, 1, 623; Olney, J. W.*Annu. Rev. Pharmacol. Toxicol.*, **1990**, 30, 47 and references cited therein.
2. Johnson, J. W., Ascher, P. *Nature*, **1987**, 325, 529.
3. For reviews, see: Palfreyman, M. G. *Excitatory Amino Acid Antagonists*, Meldrum, B. S. Ed.; Blackwell, **1991**, pp. 101-129; Bigge, C. F., Malone, T. C. *Current Opinion in Therapeutic Patents*, **1993**, 951; Leeson, P. D. *Drug Design for Neuroscience*, Kozikowski, A. P. Ed.; Raven Press, **1993**, pp. 339-381, and references cited therein.
4. The affinities of tested compounds for the glycine receptor were evaluated using the binding of [3H]-dichlorokynurenate ([3H]-DCKA) to rat cortical membranes according to Canton, T. *et al.* (*J. Pharm. Pharmacol.* **1992**, 44, 812.).
5. Mignani, S., Jimonet, P., Audiau, F., Aloup, J-C., Damour, D. *WO* 21170, **1993** (*Chem. Abstr.* 93 351607).
6. Short, J. H., Biermacher, U., *J. Am. Chem. Soc.* **1960**, 82, 1135.
7. Melting point (°C), **7a:** 260, white solid; **7b:** >250, white solid; **7c:** 219, white solid; **7d:** 83, white solid; **7e:** 290, cream-coloured solid; **7f:** 67, cream-coloured solid; **7g:** 130, sodium salt, white solid; **7h:** 257, white solid; **7i:** 224, white solid; **7j:** 130, white solid; **7k:** 211, white solid; **7l:** 224, white solid; **7m:** 221, white solid; **8a:** 95, white solid; **8b:** 80, white solid; **8c:** 129, white solid; **8d:** 110, white solid; **8e:** 90, cream-coloured solid; **8f:** 100, white solid; **8g:** 109, white solid; **8h:** 98, white solid; **8i:** 190, white solid; **8j:** 105, white solid, **8k:** 217, white solid; **8l:** 130, white solid; **8m:** 238, white solid; **8n:** 100, yellow solid; **8o:** 115, white solid, **8p:** 80, white solid; **8q:** 200, white solid; **8r:** 100, white solid; **8s:** 103, white solid; **8t:** 110, white solid; **8u:** 110, white solid; **8v:** 94, white solid; **8w:** 125, white solid.
8. Accumulation of cGMP in cerebellar slices (0.5 X 0.5 mm) from immature (9 to 12 days) rats was measured according to the method of Ferrandelli *et al.* (*Brain Res.*, **1975**, 84, 63).
9. Electrophysiological studies : NMDA was applied by microiontophoresis during extracellular unitary recording of thalamic neurons *in vivo*. Excitatory responses were quantified by measuring every 5 min the amplitude of the integrated response to NMDA. Drug effects after i.v. injection of **RPR 104632** were normalized with respect to the pre-drug baseline response.
10. Anticonvulsant activity: groups of 6 mice (DBA/2; three weeks old) received i c.v. injection (4µl) of either **RPR 104632** or vehicle by i.c.v. route (4 µl). After 15 min, mice were subjected to a high frequency noise (126 db, 12 kHz) for 60 s, which induced in control mice wild running and seizures. ED_{50} was defined as the dose of drug which totally protected 50% of the mice.
11. Recently, Merck Laboratories described 3'-(aryloxy)-3-phenyl-4-hydroxyquinolin-2(1H)-ones as potent orally active antagonists for the glycine binding site (IC_{50} [^{3}H]-L-689,560 = 1.4 - 170 nM); Kulagowski *et al. J. Med. Chem.*, **1994**, 37, 1402.

(*Received in Belgium* 31 *August* 1994)