Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 13, pp. 1529-1534, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00267-3

SUBSTITUTED MORPHOLINE-2*S*-ACETIC ACID DERIVATIVES: SCH 50911 AND RELATED COMPOUNDS AS NOVEL GABA$_B$ ANTAGONISTS

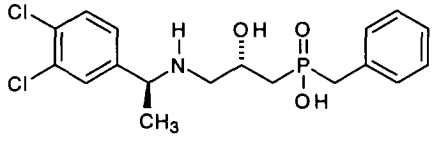
David J. Blythin,* Shen-Chun Kuo, Ho-Jane Shue, Andrew T. McPhail,¶

Richard W. Chapman,§ William Kreutner,§ Charles Rizzo,§ Hoyan S. She,§ and Robert West§

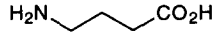
Chemical Research Department, and §Department of Allergy and Immunology, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033
¶Paul M. Gross Chemical Laboratory, Duke University, Durham, NC 27708

Abstract: The synthesis and GABA$_B$ antagonist activity of a series of substituted morpholine-2-acetic acid derivatives is described. Resolution of the lead compound from the series produces one active and one inactive enantiomer. X-ray analysis of a halogenated derivative (**25**) of the active enantiomer Sch 50911 (**23**) shows that it possesses the 2*S* configuration.

Introduction: GABA$_B$ antagonists are of potential use in the treatment of absence seizures (petit mal epilepsy).[1] In addition, there is preliminary evidence that they may be useful for the treatment of certain CNS disorders involving memory deficits, such as Alzheimer's Disease.[2] They may also be of use in countering the respiratory depression caused by excessive doses of GABA$_B$ agonists such as baclofen. The search for an effective GABA$_B$ antagonist has led to the discovery of a series of very potent phosphinic acid-derivatives (e.g., **1**) that is structurally related to the natural agonist, γ-aminobutyric acid (**2**; GABA).[3,4] An earlier generation of GABA$_B$ antagonists is illustrated by phaclofen (**3**), which is the phosphonic acid analog of baclofen (**4**).[5] Baclofen is a clinically useful GABA$_B$ agonist.[6] In our search for GABA$_B$ agonists and antagonists we synthesized and tested the unsubstituted morpholine-2-acetic acid (**9**) and found that it possessed a trace of activity in our binding assay. The subsequent development of this series is described below.



CGP 55 845; **1**



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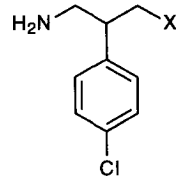
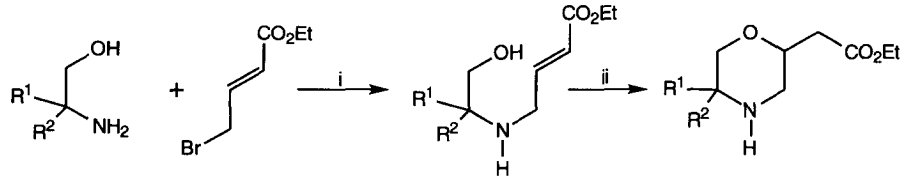


3 ; X = -P(=O)(OH)$_2$

4 ; X = -CO$_2$H

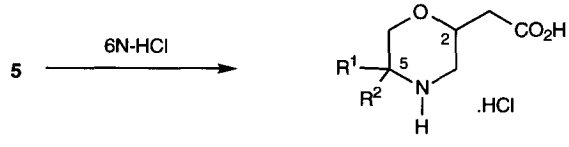
Chemistry: The morpholine-5-acetic acid ester derivatives (**5**) were synthesized according to the process shown in Scheme 1. The key step was the base-catalyzed ring closure of (**8**) to the morpholine derivative (**5**). This method of ring formation by intramolecular Michael reaction has been described recently for the synthesis of morpholine inhibitors of carnitine acetyltransferase.[7]



6 **7** **8** **5**

Scheme 1 *Reagents and conditions*: (i) Hünig's Base, CH_2Cl_2, rt, 24 h; (ii) DBU, toluene, reflux, 3-20 h.

Intermediates **8** were prepared from readily available ethyl 4-bromocrotonate **7** and the substituted aminoethanols **6**, most of which were available from commercial sources. Flash chromatographic purification of the crude **5** led to the isolation of a series of esters in yields generally in excess of 50%. The esters were hydrolyzed by heating with 6N-HCl, and the resulting solutions were evaporated under vacuum to yield the crystalline HCl salts of the final products, **9–21**. Compounds **13–15** were prepared from enantiomerically pure starting materials **6**. However, the *cis* diastereomer (2*S*,5*S*)) of **15** was formed in insufficient amount for isolation. The diastereomeric pairs of products **10** and **11**, **16** and **17**, and **18** and **19** were separated by column chromatography but ^{1}H NMR suggested that **16** still contained *ca*. 10% of its diastereomer, **17**.



9 - 21

Pharmacology and SAR: The unsubstituted derivative **9** was identified in the $GABA_B$ binding assay as a potential lead compound with modest activity (Table 1.) It showed no $GABA_B$ agonist activity in a functional in vitro assay, but showed slight activity as an antagonist of baclofen in the same assay. Inclusion of a 5-methyl group (**10** and **11**) improved binding activity slightly with no apparent difference between *cis* and *trans* diastereomers. Incorporation of two methyl groups at C5 gave **12** which showed marked improvement in binding potency. The pure enantiomers (**13**, **14**, and **15**) gave a preliminary indication that the 2*S* configuration was favored although the desired *cis* diastereomer of **15** was not available. Of the two pairs of alkyl/hydroxyalkyl substituents tested (compounds **16–19**) the diastereomer with the alkyl group *cis* to the 2-substituent was more active (**17** > **16**; **19** > **18**). The two spirocyclic

derivatives (**20** and **21**) were less active than other 5,5-disubstituted derivatives and were not examined extensively.

Compound **12** was chosen for further study, including separation into its enantiomers, and determination of the absolute configuration of the active enantiomer.

TABLE 1

Physical Properties and $GABA_B$ Antagonist Activity of Morpholine-2-Acetic Acid Derivatives (**9–21**)

Compound Number	and Stereochemistry	R^1	R^2	Yield[a] (%)	mp (°C)	$GABA_B$ Binding[b] IC_{50} (μM) ±SEM; (n)[d]	$GABA_B$ in vitro[c] Inhibn.% ±SEM;(n) at 300 μM[d]	IC_{50} (μM) (95% conf. limits)
9	2*RS*	H	H	25	172–174	>100[e]	46 (44,48)	
10	(2*RS*,5*RS*)	H	CH_3	29	165–168	9 ± 7 (3)	96 (92,100)	
11	(2*RS*,5*SR*)	CH_3	H	29	219–221	37 ± 19 (3)	76 (75,76)	
12	2*RS*	CH_3	CH_3	67	204–206	3 ± 1 (4)	84 ± 1 (3)[f,*]	4.6 (2.8–7.9)
13	(2*R*, 5*S*)	C_2H_5	H	23	140–142	>100	—	
14	(2*R*, 5*R*)	H	C_2H_5	5	148–151	>100	—	
15	(2*S*, 5*R*)	C_2H_5	H	33	140–144	10 ± 4 (3)	95 (95,95)	
16	(2*RS*,5*RS*)	CH_3	CH_2OH	30	125–128	5 ± 2 (3)	24 ± 9 (3)[f,†]	
17	(2*RS*,5*SR*)	CH_2OH	CH_3	8	181–184	2 (1.8,2.2)	74 ± 6 (3)[f,*]	4.5 (2.0–10.9)
18	(2*RS*,5*RS*)	C_2H_5	CH_2OH	28	146–149	100 (60,140)	—	
19	(2*RS*,5*SR*)	CH_2OH	C_2H_5	30	[g]	19 ± 7 (4)	99 (98,100)	
20	2*RS*	$-(CH_2)_2-$		37	188–190	37 (25,49)	—	
21	2*RS*	$-(CH_2)_4-$		14	186–188	11 ± 5 (3)	97 (95,100)	
CGP 35348						62 (49,75)	50 ± 2 (4)[f,*]	65 (39–112)

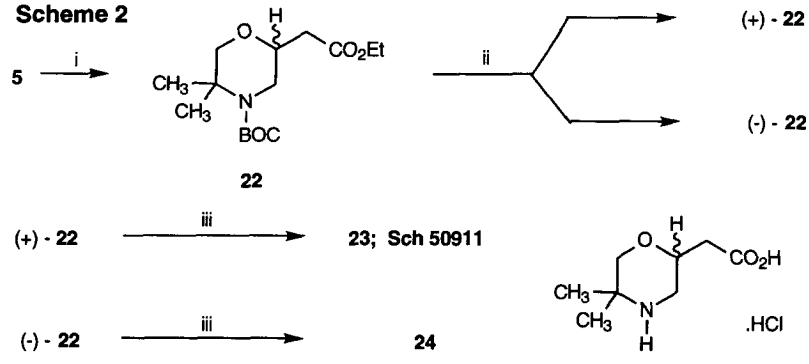
* $p < 0.05$, Student's t test; † not significant.

[a]Yield is for the two steps from **8** to the final product. [b]Preparation of rat brain synaptosomes and the assay for $GABA_B$ receptor binding were performed as described elsewhere.[8] [c]Reversal of inhibitory effects of 30 μM baclofen on EFS stimulated neuronal cholinergic contractions of guinea pig tracheal rings. Isolated guinea pig tracheal rings were incubated under a resting load of 0.5 g in 37 °C low (0.6 mM) Ca^{2+} Tyrodes buffer (pH 7.4) supplemented with 5.6 mM glucose, 30 μM choline, and 2 μM indomethacin. The preparation was continuously aerated with 95% O_2-5% CO_2. After 90 m, 5 s trains of electrical field stimulation (EFS = 20 v, 300 mA, 0.5 ms pulse duration, 8 Hz) each min gave contractions, mediated by postganglionic cholinergic neurons, that were inhibited by $GABA_B$ agonists. Antagonists were added to the bath 10 m before addition of 30 μM baclofen.[9] [d]where n = 2 results are expressed as the average, and the individual values are shown in parentheses. [e]Initial screening result gave an IC_{50} of ca. 50 μM. [f]Measurements were performed at an antagonist concentration of 30 μM.

[g]Hygroscopic - no mp taken.

Separation of 12 into Enantiomers: The intermediate **5** from Scheme 1 (in which $R^1 = R^2 = CH_3$) was separated into its enantiomers as shown in Scheme 2. Conversion of the ethyl ester to either the N-Cbz or, preferably, the N-BOC derivative **22** produced compounds that showed good separation (α = 1.54 for **22**) on a Daicel Chiralcel OD® column, eluting with hexane: 1% isopropanol. Between 250 and 500 mg of racemate could be separated cleanly in a single run. Removal of the BOC group and the ethyl ester was accomplished in a single step by dissolving each enantiomer in 6N-HCl and storing at room temperature for several days. Evaporation of the acid led to the crystalline HCl salts of the enantiomers (+)-**23** and (-)-**24**. As shown in Table 2, activity resides in the first-eluting enantiomer, (+)-**23**, known as Sch 50911. Accounts of the $GABA_B$ activity of Sch 50911 have been published along with the observation that **23** does not bind to the $GABA_A$ receptor up to at least 100μM.[8,10]

Scheme 2



Reagents and conditions: (i) $(BOC)_2O$, Hünig's Base, CH_2Cl_2, 25 °C,18 h; (ii) Daicel Chiralcel OD® 5 x 50 cm column, hexane (99%)/isopropanol (1%), 50 mL/min; (iii) 6N-HCl, 25 °C, 60 h.

TABLE 2

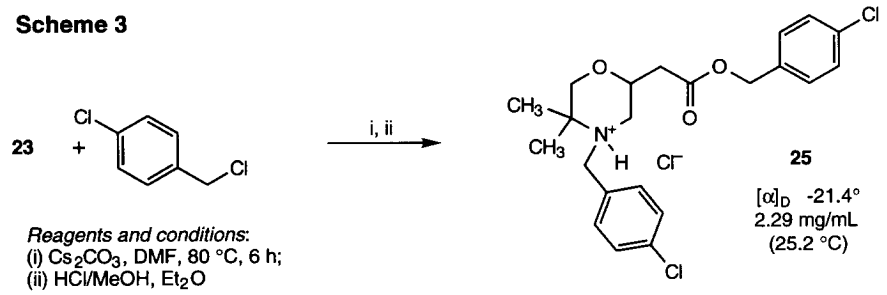
Physical Properties and $GABA_B$ Activity of Sch 50911 and its Enantiomer

Compound	Rotation $[\alpha]_D$ (H_2O)	mp (°C)	$GABA_B$ Binding IC_{50} (μM)[8]	$GABA_B$ in vitro IC_{50} (μM) (95% conf. limits)
23.HCl	+16.5° (25 °C)	154.5–157	1.1 ± 0.5	3.5 (1.8–7.0)
24.HCl	–18.7° (23 °C)	154.5–157	>100	>300

Absolute Stereochemistry of Sch 50911: Crystals of Sch 50911.HCl, **23**, suitable for X-ray analysis, proved difficult to obtain. We chose to convert a sample of **23** to a heavy atom-containing derivative. This was accomplished as shown in Scheme 3. Suitable crystals of **25**, the N-(4-chlorobenzyl), 4-chlorobenzyl ester, HCl salt derivative of **23**, were obtained by recrystallization from lower alcohol solvents. X-ray

crystallographic analysis[11] established the absolute configuration at C(2) as *S*. Fig. 1 shows the structure and solid-state conformation of **25**.

Scheme 3



Reagents and conditions:
(i) Cs_2CO_3, DMF, 80 °C, 6 h;
(ii) HCl/MeOH, Et_2O

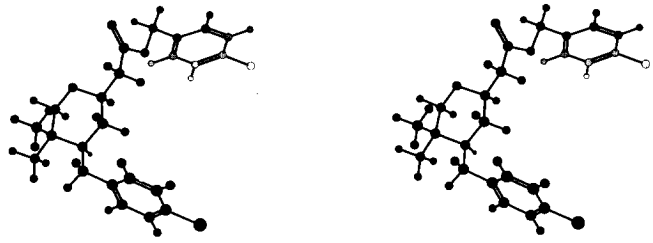


Figure 1
Stereo view from X-ray coordinates showing the structure and solid state conformation of **25**.

Conclusions: We have described the synthesis and SAR of a novel series of $GABA_B$ antagonists. The lead compound of this series, Sch 50911, **23**, has been shown to possess a range of pharmacological activities related to its $GABA_B$ activity, both in vitro and in vivo.[8,10] The observed stereochemistry at C-2 (*S*) is the opposite of that claimed for a series of highly potent phosphinic acid antagonists, such as **1**.

Experimental: Preparation of Sch 50911 (23). To a solution of 2-amino-2-methyl-1-propanol (1.8 g) in CH_2Cl_2 (40 mL) was added ethyl 4-bromocrotonate (3.3 mL). Hünig's Base (5.2 mL) was added, and the mixture was stirred at rt (ca. 23 °C) for 24 h. Solvent was removed under vacuum, the residue was suspended in EtOAc (60 mL), and was stirred for 0.5 h. The mixture was filtered and evaporated. The crude product was purified by flash chromatography (silica gel, CH_2Cl_2:MeOH/NH_3, 95:5) to yield 2.5 g (65%) of **8** ($R^1 = R^2 = CH_3$; mp 71–73 °C.) In toluene (50 mL) was dissolved **8** ($R^1 = R^2 = CH_3$; 1.78 g) and DBU (0.18 g.) The solution was heated to reflux and kept there for 18 h. After cooling the reaction mixture, solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica gel, CH_2Cl_2:MeOH/NH_3, 95:5) to yield **5** ($R^1 = R^2 = CH_3$) as an oil. 1 g of **5** was dissolved in CH_2Cl_2 (10 mL) at rt. A solution of di-t-butyl dicarbonate ("BOC anhydride" 1.2 g) in CH_2Cl_2 (3 mL) was added followed, about 0.5 h later, by a few drops of Hünig's base. The solution was allowed to stand at rt overnight. The reaction mixture was concentrated under vacuum and the residue was dissolved in EtOAc. This solution was washed with brine, dried (Na_2SO_4), filtered, and evaporated to yield **22**, mp 36–38 °C. A 10% solution of **22** was made up in hexane:isopropanol (95:5) and between 2.5 and 5.0 mL of this solution was introduced on to a Daicel ChiralCel OD® 5 x 50 cm preparative HPLC column which was being run with hexane:isopropanol (99:1). The enantiomers were completely separated under these conditions in just under 1 h at a flow rate of 50 mL/min. The α-value was ca. 1.54. The first eluting enantiomer (**22**; mp 56–58 °C; $[\alpha]_D$ +21.8° (23 °C, MeOH); 1.2 g) was suspended in 6N-HCl (12 mL) at rt under N_2. The mixture eventually became a clear solution. After 48 h water (2 mL) was added and the aqueous solution was extracted with EtOAc (3 x 5 mL.) The aqueous layer was filtered and evaporated to yield **23**.HCl as a white solid, 0.75 g (84%; mp 154.5–157 °C; $[\alpha]_D$ = +28.6° (21 °C, MeOH)).

Acknowledgments: We thank Ms. X. Chen and Ms. Y. Chen for performing several of the experimental procedures, and the staff of the Physical and Analytical Chemistry Research Department of the Schering-Plough Research Institute for acquiring the analytical data.

References and Notes:

1 (a) Hosford, D. A.; Clark, S.; Cao, Z.; Wilson, W. A.; Lin, F.-H.; Morrisett, R. A.; Huin, A. *Science* **1992**, *257*, 398; (b) Snead, O. C. *Eur. J. of Pharmacol.* **1992**, *213*, 343.

2 Mondadori, C.; Preiswerk, G.; Jaekel, J. *Pharmacol. Commun.* **1992**, *2*, 93.

3 Froestl,W.; Mickel, S. J.; von Sprecher, G.; Diel, P. J.; Hall, R. G.; Maier, L.; Strub, D.; Melillo, V.; Baumann, P. A,; Bernasconi, R.; Gentsch, C.; Hauser, K.; Jaekel, J.; Karlsson, G.; Klebs, K.; Maître, L.; Marescaux, C.; Pozza, M. F.; Schmutz, M.; Steinmann, M. W.; van Riezen, H.; Vassout, A.; Mondadori, C.; Olpe, H.-R.; Waldmeier, P. C.; Bittiger, H. *J. Med. Chem.* **1995**, *38*, 3313.

4 Froestl, W.; Mickel, S. J.; von Sprecher, Bittiger, H.; Olpe, H.-R. *Pharmacol. Commun.* **1992**, *2*, 52.

5 (a) Chiefari, J.; Galanopoulos, S.; Janowski, W. K.; Kerr, D. I. B.; Prager, R. H. *Austral. J. Chem.* **1987**, *40*, 1511; (b) Kerr, D. I. B.; Ong, J.; Prager, R. H.; Gynther, S. D.; Curtis, D. R. *Brain Res.* **1987**, *405*, 150.

6 For example, Standaert, D. G., Young, A. B. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th Edition; Hardman, J. G.; Limbird, L. E., Eds.; McGraw-Hill: New York, 1996; p 517; *Physicians' Desk Reference*, 49th Edition; Medical Economics Data Production Company: 1995; p 1065.

7 Sun, G.; Savle, P. S.; Gandour, R. D.; a'Bhaird, N. N.; Ramsay, R. R.; Fronczek, F. R. *J. Org. Chem.* **1995**, *60*, 6688.

8 Bolser, D. C.; Blythin, D. J.; Chapman, R. W.; Egan, R. W.; Hey, J. A.; Rizzo, C.; Kuo, S.-C.; Kreutner, W. *J. Pharmacol. and Exper. Ther.* **1995**, *274*, 1393.

9 Chapman, R. W.; Danko, G.; del Prado, M.; Egan, R. W.; Kreutner, W.; Rizzo, C. A.; Hey, J. A. *Pharmacology* **1993**, *46*, 315.

10 (a) Hosford, D. A.; Wang, Y.; Liu, C. C.; Snead, O. C. *J. Pharmacol. and Exper. Ther.* **1995**, *274*, 1399; (b) Cunningham, M. D.; Enna, S. J. *Brain Research*, in press.

11 *Crystal data for* **25**: $C_{22}H_{26}Cl_3NO_3$, M = 458.82, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 12.886(2) Å, b = 25.683(4) Å, c = 6.933(1) Å, V = 2295(1) Å^3, Z = 4, $D_{calcd.}$ = 1.328 g cm^{-3}, μ(Cu-Kα radiation, λ = 1.5418 Å) = 38.7 cm^{-1}. Intensity data ($+h,+k,+l$; 2714 reflections, $\theta_{max.}$ = 75°) were recorded on an Enraf-Nonius CAD-4 diffractometer [Cu-Kα radiation, graphite monochromator; ω-2θ scans, scanwidth (1.15 + 0.14tanθ)°] from a crystal of dimensions 0.18 x 0.36 x0.40 mm. The crystal structure was solved by direct methods (MULTAN11/82). The absolute configuration was determined by use of anomalous scattering effects. Full-matrix least-squares refinement of atomic positional and thermal parameters (anisotropic C, Cl, N, O; fixed H contributions) converged (max. shift:esd = 0.03) at R = 0.047 (R_w = 0.063) over 1938 absorption-corrected [$T_{max.}$:$T_{min.}$(rel.) = 1.00:0.63] reflections with I>3.0σ(I). Atomic parameters, bond lengths, bond angles and torsion angles for **25** have been deposited at the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, U.K.

(*Received in USA* 19 *April* 1996; *accepted* 6 *June* 1996)