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OPTICAL ISOMERS OF RP 64406: NEW POTENT ANTIGLUTAMATE AGENTS

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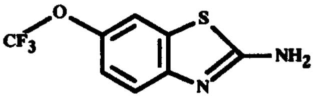
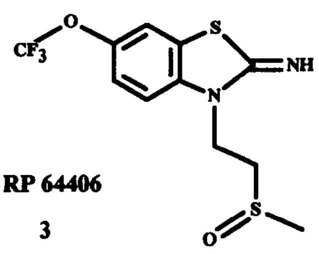
Abstract: Optical isomers of the riluzole derivative, sulfoxide RP 64406, were prepared in pure forms. No significant difference was found between the (+) and (-) isomers in their powerful antiglutamate activity.

The serendipitous discovery in our laboratories of *in vivo* antiglutamate[1a] and neuroprotective[1b] activities of riluzole 1 (6-trifluoromethoxy-2-benzothiazolamine, RP 54274, PK 26124), led us to develop a comprehensive program of analogues with the objective to identify a second generation of more potent antiglutamate and antiischaemic agents. However, the antiglutamate activity of riluzole does not appear to involve a direct interaction with excitatory amino acid receptors,[1c] but rather modulation of glutamic acid release by riluzole, possibly by inactivation of sodium channels.[1d] We have therefore chosen a functional assessment of antiglutamate activity, antagonism of glutamate-evoked convulsions in rats.[2]

The synthesis of various 3-substituted-6-trifluoromethoxy-2-imino-benzothiazolines 2[3] showed that strong protective effects against these convulsions required the presence of 3-substituents containing, either a nitrogen atom, or an unchanged or oxidized sulfur atom.[4]



riluzole 1



RP 64406

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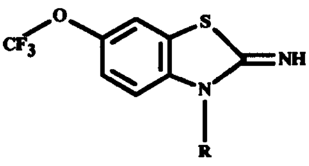


2 R = $(CH_2)_n NR_1R_2$
R = $(CH_2)_n S(O)_m R_3$

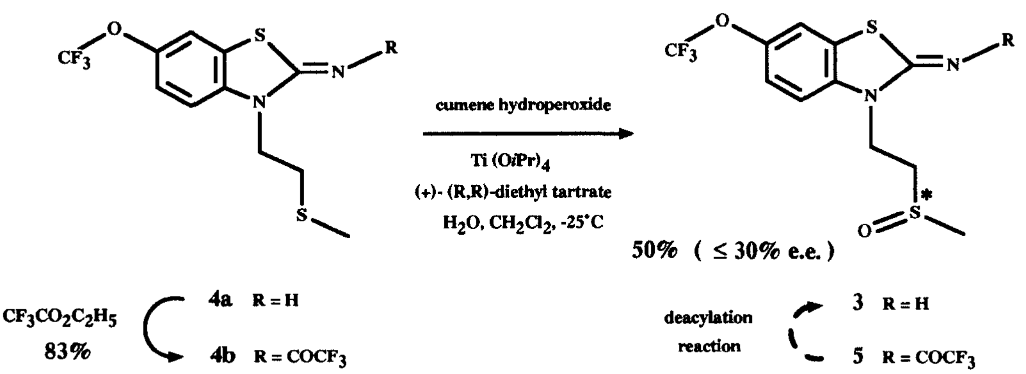
Optimization of this series of benzothiazolines led to the synthesis of the sulfoxide RP 64406 3, which displayed a three times more potent protection than riluzole against glutamate-induced convulsions in rats (Table 2). These results prompted us to prepare each enantiomer of RP 64406.

In this communication we report a high-yielding preparation of the pure enantiomers 3(+) and 3(-) of RP 64406 and our preliminary biological investigations in their *in vivo* antiglutamate activity.

Initial studies were carried out by attempting a straightforward preparation of pure enantiomers of RP 64406, using chiral phase preparative chromatographic separation of the racemic compound. The analytical resolution of RP 64406 was performed by HPLC using Chiralcel OD (Daicel) as chiral stationary phase.[5] Unfortunately, this separation was not scaled up due to poor resolution and solubility.

Synthesis of enantiomers 3(+) and 3(-) was then endeavoured by direct asymmetric oxidation of the methylsulfide group of compound **4a**[6] to the corresponding sulfoxide under KAGAN's procedure ($Ti(O\text{-}i\text{-}Pr)_4$/(+)-(R,R)-diethyl tartrate/cumene hydroperoxide/H_2O = 1/2/1/1, Scheme 1).[7] This asymmetric oxidation showed a maximum e.e. value (enantiomeric excess) of 30%[8] and the oxidized compound 3 was obtained in 50% yield. Under identical conditions, the same yield and e.e. were obtained when using acylated compound **4b**[9] to give 5,[10] a precursor of 3. However, low e.e.'s made this approach unattractive for the synthesis of pure enantiomers of RP 64406.

Scheme 1 : asymmetric oxidation of compounds 4a and 4b



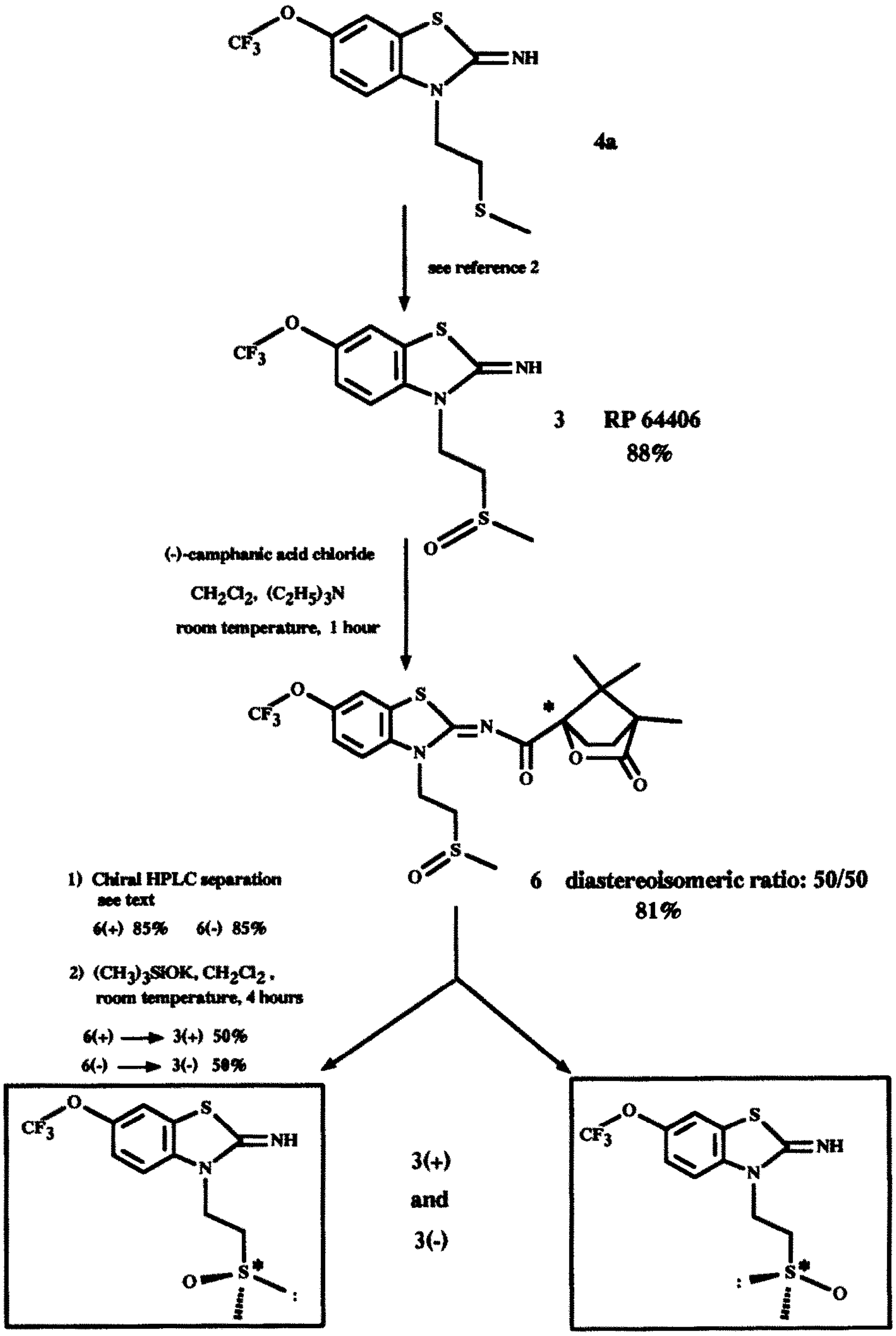
Finally, the synthesis of the pure enantiomers of RP 64406, 3(+) and 3(-), was achieved in a four step procedure starting from **4a** (Scheme 2).

Oxidation of **4a** by 3-chloroperoxybenzoic acid in ethanol provided RP 64406 in 88% yield.[3] This racemic sulfoxide reacted with (-)-camphanic acid chloride in dichloromethane at room temperature in presence of triethylamine to give a mixture of diastereoisomers 6 in 81% isolated yield; ^{1}H-NMR analysis indicated a 50/50 molar ratio[11]. Each diastereoisomer was isolated in optically pure form[12] by HPLC using a column packed with cellulose trisphenylcarbamate-coated silicagel. Eight runs (recycling included) were necessary to separate 1.4g of starting compound 6 (mobile phase: ethanol, flow-rate: 10ml/mn, pressure: 15 bars, Table 1)[13]. No preparative chromatographic HPLC separation of the diastereoisomers of 6 was observed using standard silicagel as stationary phase[14].

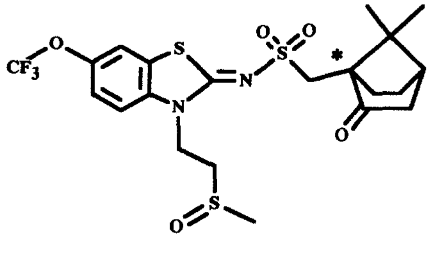
Table 1: chromatographic separation of diastereoisomers 6 (+) and 6 (-)

	retention time	weight	yield	$[\alpha]_D^{20}$
1st eluted diastereoisomer 6 (+)	15.1 mn	0.592 g	85%	+ 36.6° ± 0.8° (c=1, EtOH)
2nd eluted diastereoisomer 6 (-)	22.2 mn	0.595 g	85%	- 54.9° ± 1° (c= 1, EtOH)

Scheme 2: Preparation of RP 64406 enantiomers 3(+) and 3(-)



In addition, a weak diastereodifferentiation was observed with compound **7** in preparative HPLC using cellulose trisphenylcarbamate/silica gel as stationary phase .



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Both diastereoisomers **6**(+) and **6**(-) were readily converted into the desired enantiomers **3**(+) and **3**(-) in optically pure form by a clean deacylation reaction under mild conditions using potassium trimethylsilanolate as nucleophilic agent. Enantiomers were obtained in a 50% yield.[15] To the best of our knowledge, such useful deacylation using this silylated compound has not yet been described.[16]

Enantiomeric homogeneity of both enantiomers (>99% e.e.) was evaluated by HPLC using Chiralcel OD as chiral stationary phase[5] and by ^{1}H-NMR analysis using (R)-(-)-N-(3,5-dinitrobenzoyl)-α-methylbenzylamine as chiral reagent.[17] Optical rotation $[\alpha]_D^{20}$ (c=1, EtOH) was +57° for **3**(+) and -59° for **3**(-).

Both enantiomers were tested for their *in vivo* ability to protect against glutamate-induced convulsions in rats;[2] antiglutamate activity is reported in table 2.

Table 2: ***in vivo* antiglutamate activity**

Compounds	glutamate-induced convulsions ED_{50} mg/kg i.p.
riluzole **1**	3.2
RP 64406 **3**	1.1
enantiomer **3** (-)	1.6
enantiomer **3** (+)	1.0

The two enantiomers displayed similar antiglutamate activity; this result is certainly due to the flexibility of the methylsulfinylethyl chain and suggests that the binding site can accommodate either R- or S-methylsulfinylethyl side chain. However, a metabolic process leading to thioether **4a** (ED_{50}= 1.0 mg/kg i.p.) cannot be ruled out.[18] Biochemical, cerebral antiischaemic studies and side effect evaluations of each enantiomer of RP 64406 are under investigations and will be reported soon.

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References and Notes.

1. a- Mizoule, J.; Meldrum, B.; Mazadier, M.; Croucher, M.; Ollat, C.; Uzan, A.; Legrand, J-J.; Gueremy, C.; Le Fur, G. *Neuropharmacology* **1985**, *24*(8), 767.
   b- Pratt, J.; Rataud, J.; Bardot, F.; Roux, M.; Blanchard, J-C.; Laduron, P.M.; Stutzmann, J-M. *Neuroscience Letters* **1992**, *140*, 225 and references cited therein.
   c- Benavides, J.; Camelin, J.C.; Mitrani, N.; Flamand, F.; Uzan, A.; Legrand, J-J.; Gueremy, C.; Le Fur, G. *Neuropharmacology* **1985**, *24*(11), 1085.
   d- Benoit, E.; Escande, D. *Pflugers Arch.* **1991**, *419*(6), 603.

2. Antiglutamate activity of studied compounds was carried out according to the following method: Groups of 7 male Sprague-Dawley rats weighing about 200g were used. Drugs were injected i.p. 30 minutes prior to intracerebroventricular (i.c.v.) administration of 10 µl/rat of a 12.5 µmol/kg solution of L-glutamate in saline according to a free hand technique. After this treatment, the rats were observed for 30 minutes; L-glutamate alone provoked seizures immediately after injection: animals ran wildly, jumped violently and exhibited clonic seizures. ED_{50} for studied compounds was defined as the dose of drug (mg/kg) which totally protected 50 per cent of the rats from clonic convulsions.

3. Gueremy, C.; Jimonet, P.; Mignani, S. *EP 374,040*, **1990**; *Chem. Abstr.* **1991**, *113*, 231363p.

4. Presented in part at: XIIth International Symposium on Medicinal Chemistry, Basel, Switzerland, 1992; Jimonet, P.; Audiau, F.; Barreau, M.; Beaudoin F.; Blanchard, J-C.; Dutruc-Rosset, G.; Gueremy, C.; Laduron, P.M.; Le Blevec, J.; Mignani, S.; Nemecek, C.; Rataud, J.; Stutzmann, J-M.

5. Retention times were 36 mn and 41 mn for 3(+) and 3(-) respectively (column: Chiralcel OD (Daicel); mobile phase: ethanol/*n*-hexane = 5/95: v/v; flow rate: 1ml/mn; baseline resolution: R_s=1.9).

6. Compound **4a** was prepared in a one-step synthesis in 82 % yield by direct alkylation of riluzole with 2-chloroethylmethyl sulfide (see reference 3).

7. Zhao, S.H.; Samuel, O.; Kagan, H.B. *Tetrahedron*, **1987**, *43* (21), 5135.

8. Enantiomeric purity was based on the variation of the optical rotation from successive fractional cristallizations of **3**.

9. Compound **4b** (m.p.= 125°C) was prepared by reaction of **4a** with ethyl trifluoroacetate and triethylamine in ethanol for 72 hours at room temperature (83% yield). Satisfactory spectral data have been obtained for **4b**.

10. Enantiomeric purity of **5** was based on the ^{1}H-NMR experiments using (R)-(-)-N-(3,5-dinitrobenzoyl)-α-methyl-benzylamine (Aldrich) as chiral resolving agent (1/1 molar ratio).
    The diastereoisomeric 65/35 molar ratio is based on integration of the ^{1}H-NMR peak signals of the $SOCH_3$ at δ= 2.70 and 2.72 ppm.
    ^{1}H-NMR ($CDCl_3$, 300MHz) δ: 2.70 and 2.72 (two s, 3H, $SOCH_3$), 3.15 and 3.43 (two m, 2X1H, $-SOCH_2$), 4.93 (m, 2H, CH_2N), 7.5 (bd, J=9Hz, 1H, H-5), 7.67 (bs, 1H, H-7), 7.83 (d, J=9Hz, 1H, H-4).

11. *Experimental procedure:* (-)-Camphanic acid chloride (Fluka, 2.16g, 10 mmol) was slowly added to an anhydrous solution of RP 64406 (3.24g, 10 mmol) and triethylamine (1.4 ml, 10 mmol) in dichloromethane (30 ml). The resulting mixture was stirred for 1 hour at room temperature and water (50 ml) was added. After extraction with dichloromethane (3 X 50 ml), the combined organic layers were dried over magnesium sulphate, filtered, and concentrated to dryness. The residu was purified by flash chromatography using ethyl acetate as eluent to furnished a 50/50 molar ratio of diastereoisomers **6** in 81% yield as a white solid [4.1 g, white semi-solid, R_f(one spot)= 0.15 in ethyl acetate on silica gel (Merck Kieselgel $60F_{254}$)].
    The diastereoisomeric ratio is based on the integration of the ^{1}H-NMR peak signals of $OCO-C-CH_3$ at δ= 1.17 and 1.15 ppm and $-SOCH_3$ at δ= 2.62 and 2.65 ppm (see reference 12).

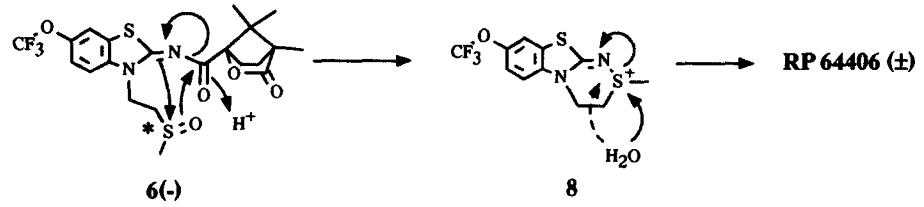
12. Diastereoseparation by preparative HPLC gave compounds **6**(+) and **6**(-).
6(+): white semi-solid; ^{1}H-NMR (DMSO-d_6, 200MHz) δ: 0.84 and 1.04 (two s, 2x6H, $C(CH_3)_2$), 1.17 (s, 3H, O-CO-C-CH_3), 1.6, 1.9 and 2.8 (three m, respectively 1H, 2H and 1H, C-CH_2-CH_2-C), 2.62 (s, 3H, $SOCH_3$), 3.15 and 3.35 (two m, 2X1H, CH_2SO), 4.83 (m, 2H, CH_2N), 7.63 (bd, J=9Hz, 1H, H-5), 7.9 (d, J=9Hz, H-4), 8.17 (bs, 1H, H-7).
6(-): white semi-solid; ^{1}H-NMR (DMSO-d_6, 200MHz) δ: 0.84 and 1.04 (two s, 2x6H, $C(CH_3)_2$), 1.15 (s, 3H,O-CO-C-CH_3), 1.6, 2.0 and 2.7 (three m, 1H, 2H and 1H respectively, C-CH_2-CH_2-C), 2.65 (s, 3H, $SOCH_3$), 3.15 and 3.35 (two m, 2X1H, CH_2SO), 4.83 (m, 2H, CH_2N), 7.63 (bd, J=9Hz, 1H, H-5), 7.9 (d, J=9Hz, H-4), 8.17 (bs, 1H, H-7).
Satisfactory mass spectra and IR data have been obtained for **6**, **6**(-) and **6**(+).

13. The chiral stationary phase was prepared from Nucleosil silicagel 1000 A - 10 µm (Macherey - Nagel) and cellulose trisphenylcarbamate, according to the procedure described by Y. Okomoto (*J. Am. Chem. Soc.*, **1984**, *106*, 357).
36g of the chiral phase was packed into a 15 cm X 2.2 cm I.D. stainless steal column by the usual slurry technique under 100 bars, using ethanol as pumping solvent.

14. No HPLC separation of the diastereoisomers of **6** was observed using standard silicagel and acetonitrile/chloroform:20/80(v/v) or cyclohexane/ethyl acetate/2-propanol:45/45/10(v/v/v) as solvents.

15. *Experimental procedure:* Potassium trimethylsilanolate (Aldrich, 0.18g, 1.4 mmol) was slowly added under nitrogen to a solution of **6**(+) (0.33g, 0.65 mmol) in dichloromethane (15 ml). The mixture was stirred at room temperature for 4h. Water (30 ml) was then added and the solution was extracted with dichloromethane (2 X 50 ml). The combined extracts were washed with water (20 ml), dried over magnesium sulphate, and evaporated to give chemically pure **3**(+) (white solid, m.p.=126°C) in 50% yield. The second enantiomer **3**(-) (white solid, m.p.=130°C) was obtained with an identical yield from the second diastereoisomer **6**(-) under similar experimental conditions.

16. Treatment of **6**(-) or **6**(+) with HCl/MeOH (room temperature, 40 h) according to the procedure described by V. Skaric (*Helv. Chim. Acta*, **1976**, *59*, 2972) gave the racemized RP 64406, whereas no racemization was observed with the non-acylated derivative **3**(-) using the same treatment. This racemization can be reasonably explained through the sulfoxide involvement in the imine deacylation reaction followed by ring opening of the cyclic sulfonium intermediate **8** with water.



Treatment of compound **6**(-) with aqueous NaOH or H_2N-NH_2 (EtOH, room temperature, 15h) afforded tars, and with NH_4OH/MeOH (reflux, 15h) no reaction was observed.

17. By using (R)-(-)-N-(3,5-dinitrobenzoyl)-α-methyl-benzylamine as chiral ^{1}H-NMR resolving agent, only one $SOCH_3$ signal was observed for compounds **3**(+) and **3**(-); in the same conditions, a 1/1 molar ratio of two $SOCH_3$ peaks was displayed by the racemic RP 64406 **3**(±): ^{1}H-NMR ($CDCl_3$, 250MHz) δ= 2.64 and 2.66 (two s, 3H, $SOCH_3$), 3.02 and 3.30 (two m, 1H and 1H, SO-CH_2), 4.4 (m, 2H, CH_2N), 7.0-7.25 (m, 3H, H-4, H-5 and H-7). Satisfactory mass spectra and IR data have been obtained for **3**(±), **3**(-) and **3**(+).

18. For an example of *in vivo* equilibrium between a sulfoxide and the corresponding thioether see: Duggan, D.E.; Hooke, K.F.; Noll, R.M.; Hucker, H.B. and Van Arman, C.G. *Biochem. Pharmacol.* **1978**, *27*, 2311.