Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 319–322

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antihypertensive Activity of Substituted 2,3,8,8a-Tetrahydro-7*H*-oxazolo[3,2-*a*]pyridinedicarboxylate Enantiomers

Elena Martín,[a] Asunción Morán,[a] Mª Luisa Martín,[a,*] Luis San Román,[a] Pilar Puebla,[b] Manuel Medarde,[b] Esther Caballero[b] and Arturo San Feliciano[b]

[a]*Laboratorio de Farmacología, Facultad de Farmacia, Universidad de Salamanca, 37007-Salamanca, Spain*
[b]*Laboratorio de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Salamanca, 37007-Salamanca, Spain*

Received 5 July 1999; accepted 9 December 1999

Abstract—The synthesis and antihypertensive activity of racemates and enantiomers of substituted 2,3,8,8a-tetrahydro-7*H*-oxazolo[3,2-*a*]pyridinedicarboxylates have been reported.

In previous studies, we prepared and showed the antihypertensive activity of several 2,3,8,8a-oxazolo[3,2-*a*]-pyridine, 2,3,8,8a-thiazolo[3,2-*a*]pyridine and 3,4,9,9a-pyrido[2,1-*b*]oxazine derivatives (**I**, **II**, **III**).[1–5] Ip administration of some of these compounds to conscious, spontaneously-hypertensive rats has displayed a long-lasting activity which, in general, was not accompanied by reflex tachycardia. Two oxazolo[3,2-*a*]pyridine and two pyrido[2,1-*b*]oxazine derivatives containing methyl or ethyl ester moieties were the most potent compounds with longer antihypertensive activity. The evaluation of these products was carried out with racemic mixtures and, with the aim of determining the activity of each enantiomer, we have now prepared and evaluated each enantiopure stereoisomer.

It has been reported that calcium antagonists, such as verapamil and diltiazem, and some 1,4-dihydropyridines, such as nicardipine and nimodipine, have marked stereoselectivity for blocking calcium entry into the cell.[6–10] 4-Aryl-1,4-dihydropyridine derivatives in particular, possessing different substituents at positions C_3 and C_5 and/or at positions C_2 and C_6 and consequently a chiral center at position C_4, showed a remarkable difference between both enantiomers in their respective blocking activities.[11–13]

This paper reports the preparation of several 2-methyl-2,3,8,8a-tetrahydrooxazolo[3,2-*a*]pyridines and the comparative pharmacological evaluation of racemates and pure enantiomers.

Chemistry

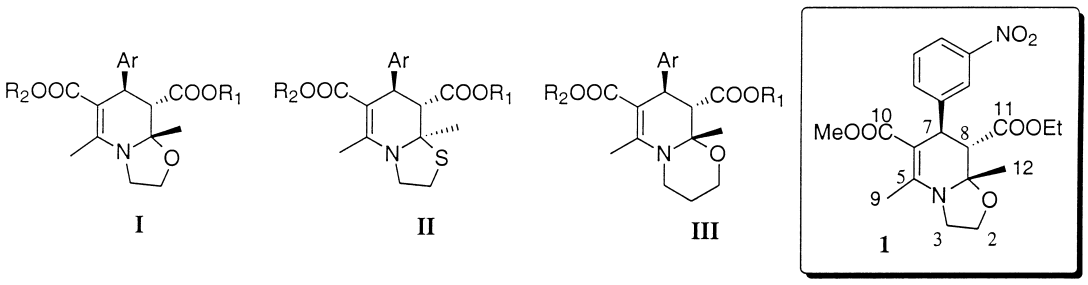
The asymmetric synthesis of both enantiomers of 1,4-dihydropyridines has been carried out by several methods including resolution of racemic monocarboxylic acid through the formation of diastereomeric salts,[14] enantioselective Hantzsch synthesis using chiral auxiliaries,[15] and chemoenzymatic synthesis of chiral derivatives.[16,17]

Years ago we tried the resolution of racemic *ethyl 7-(3-nitrophenyl)-5,8a-dimethyl-6-methoxycarbonyl-2,3,8,8a-tetrahydro-7*H*-oxazolo[3,2-a]pyridine-8-carboxylate* **1**, by two different approaches. The resolution was attempted through the formation of diastereomeric salts or esters and by enzymatic resolution. None of these processes gave adequate results, and the starting racemic mixture was recovered.[18]

As these methodologies did not allow us to obtain enantiopure forms of **1**, we designed another approach to synthesize the 2-methyl analogues of **1** based on the enantioselective synthesis that implied the control of the stereochemistry by the presence of a chiral atom in the starting enaminone. From (*2R*) or (*2S*)-1-amino-2-propanol and methyl acetylacetate the chiral enaminones

*Corresponding author. Tel.: +34-23-294-530; fax: +34-923-294-515; e-mail: marisam@gugu.usal.es

0960-894X/00/$ - see front matter
PII: S0960-894X(99)00691-5



3r or **3s** were obtained and used for synthesis of the oxazolopyridines **4r** or **4s**. As we have previously described,[19,20] the absolute stereochemistry of **4r** was confirmed by X-ray diffraction and it is in agreement with that expected from the starting (*2R*)-1-amino-2-propanol.

Following this methodology,[19] we have now prepared compounds **5r** and **5s** in 40–48% yield.[21] The absolute stereochemistries were assigned in comparison with the previous results obtained by us and the enantiomeric purity (ee >97%) of these derivatives has been checked using chiral lanthanide shift reagents by ^{1}H NMR spectroscopy evaluation.

Antihypertensive activity in conscious animals

Male adult, spontaneously-hypertensive rats (SHR aged 20–30 weeks) from the Animalarium of the University of Salamanca (Animalario Departamental, USAL, No. P.A.E.-SA001), were used in these experiments. Two days before the experiment, a polyethylene (PE-50) cannula was implanted in the left carotid artery under anesthesia with sodium pentobarbital (40 mg/kg, ip). The cannula was exited subcutaneously at the back of the neck. After cannulation, animals were housed separately, with food and water freely available.

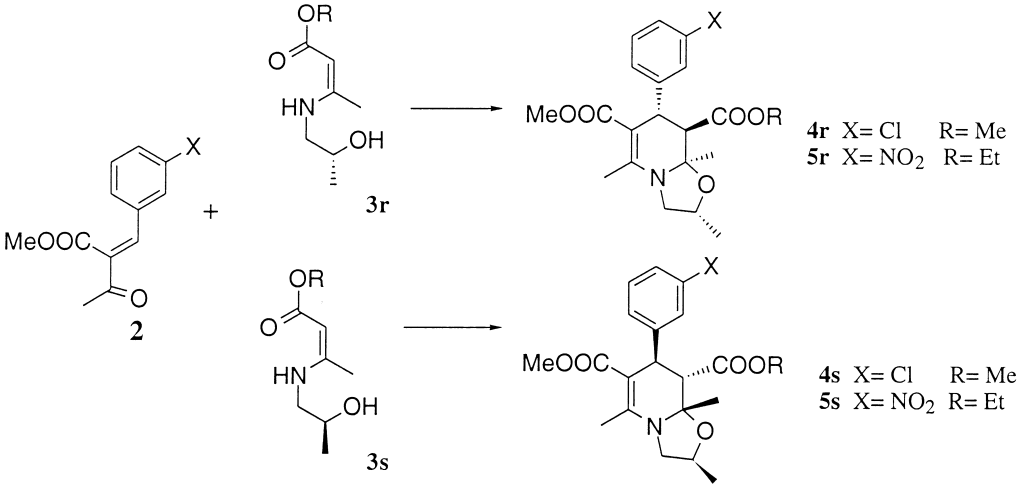
After 48 h, blood arterial pressure (BP) was measured using a Letica pressure transducer connected to a PRS 205 amplifier of a Letica Polygraph series 4000. Heart rate (HR) was measured by a CAR 1000 cardiotachometer triggered by the arterial pressure pulse. After a period of 15 min, when BP and HR reached equilibrium, each compound was injected ip as a single dose. Mean blood pressure and HR were monitored at 15 and 30 min, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after administration. Each animal was used for only one experiment.

The evaluation was performed by comparing doses of 5 mg/kg (0.014 mmols/kg) of nifedipine, 50 mg/kg (0.12 mmols/kg) of compound **1** and 50 mg/kg (0.13 mmols/kg) of the racemic **4, 5** and their pure enantiomers. The intraperitoneal administration was chosen as a rapid means of administration.

The compounds assayed were dissolved in an aqueous solution of carboxymethylcellulose and Tween 80, both at 0.1%. This vehicle was administered (5 mL/kg) to the control group. Nifedipine (Lab. Dr. Andreu) was used as the reference drug.

Expression and analysis of results

Modifications were expressed as mean values of mean arterial blood pressure (MAP, mm Hg) or HR (beats per min). All data were expressed as the mean value ± SEM of six experiments. Statistical evaluation was carried out using Student's *t*-test for unpaired values. The values displayed in figures as $*P < 0.05$ were significantly different from the control group.



Results and Discussion

The values of MAP and HR in SHR rats, before drug administration, were 164.6 ± 2.8 mm Hg and 355.5 ± 7.6 beats/min, respectively ($n = 48$). The vehicle used in our experiments (carboxymethylcellulose and Tween 80, both at 0.1%), did not cause significant changes in MAP and HR (control group in figures).

Changes in MAP, after ip administration of nifedipine or compounds **4** and **5** and their corresponding enantiomers, are shown in the Figures 1 and 2.

Compound **4** provided a modest reduction in mean arterial blood pressure but was toxic to one of six animals, where death occurred 24 h after ip dosing. Intraperitoneal administration of 50 mg/kg of the **4r** enantiomer, that resulted in a relative acute toxicity, significantly decreased mean arterial blood pressure values (Fig. 1). With this enantiomer, two of six rats died 3 h after ip administration. In all cases, death was preceded by convulsions and marked hypotension. The higher toxicity of this enantiomer, in comparison with the racemate, was also observed at a lower dose of 25 mg/kg (results not included). The **4s** enantiomer was inactive and did not show toxicity.

Based on these initial results, it is plausible that a relationship between stereoselectivity and toxicity exists for compound **4**, and that the hypotension induced by the **4r** enantiomer could be a consequence of its acute toxicity and not a specific antihypertensive effect.

In contrast to compound **4**, no toxic effect was observed for the doses of compound **5** and their corresponding enantiomers evaluated. It appears that introduction of a Cl substituent in the aromatic ring, relative to the typical NO_2 group on these compounds, increases liposolubility

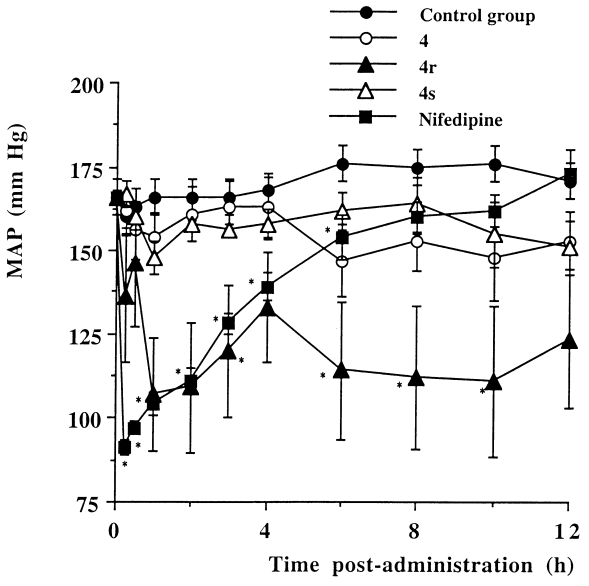


Figure 1. Effect of ip administration of vehicle (4 mL/kg), compounds **4**, **4r**, **4s** (50 mg/kg) and nifedipine (5 mg/kg), on mean arterial blood pressure (MAP) in conscious SHR rats. $^*P < 0.05$ versus control group.

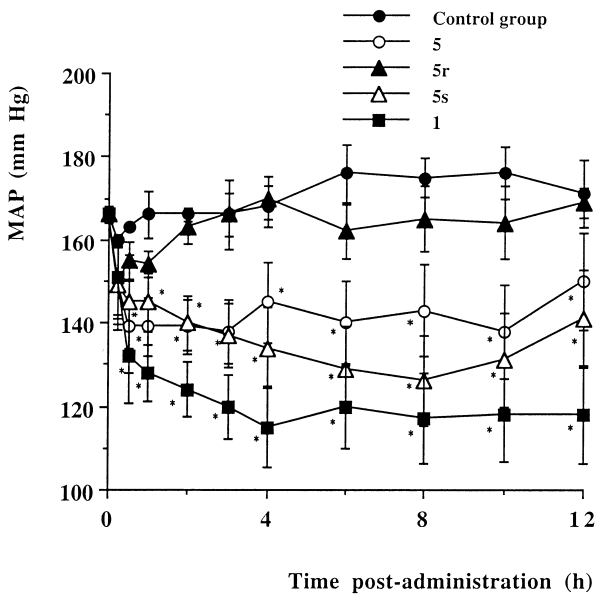


Figure 2. Effect of ip administration of vehicle (4 mL/kg), compounds **5**, **5r**, **5s** and **1** (50 mg/kg), on mean arterial blood pressure (MAP) in conscious SHR rats. $^*P < 0.05$ versus control group.

and central toxicity. In previous studies, we also observed a major acute toxicity for other oxazolopyridine and pyridoxazine derivatives having a 3-chlorophenyl substituent and no toxic effect for **1** and other nitro derivatives.[5]

Product **5**, at a dose of 50 mg/kg ip, produced a significant decrease in mean arterial blood pressure with a similar profile to that previously observed by us for compound **1** (Fig. 2). Both compounds produced a maximum reduction in MAP at 3–6 h. However, the antihypertensive activity was higher for compound **1** than compound **5**. The hypotension of racemic **5** was not accompanied by reflex tachycardia (results not included).

From the comparison of antihypertensive activity observed after ip administration of compounds **1** and **5**, we can deduce that the incorporation of a methyl group decreased the potency of this oxazolopyridine derivative.

The activity of the two enantiomers of **5** was very different. Accordingly, the **5s** enantiomer was a more active antihypertensive agent than the racemate, whereas the **5r** enantiomer was practically inactive (Fig. 2) at the dose of 50 mg/kg.

In contrast to nifedipine, the hypotensive effect induced by the compounds **1**, **5** and **5s** was gradual and maintained, with a maximum in the 3–6 h range and it remained so even after a 12–24 h interval after ip administration. Nifedipine, at the dose used by us, produced a maximum reduction in MAP at 15–30 min and the effect disappeared 4–6 h after administration (Fig. 1). The antihypertensive effect exhibited by nifedipine was accompanied by a significant reflex tachycardia (results not included). In contrast, compound **5** did not induce reflex tachycardia.

The inactivity of **5r** as an antihypertensive agent and the higher activity of **5s** relative to **5**, provide confirmation that stereochemical features of these molecules plays a decisive role in their pharmacological responses[22] which has been demonstrated for some calcium entry blockers,[23–25] is also operative in the case of the antihypertensive oxazolopyridines described in this study.

Acknowledgements

The authors would like to thank DGICYT (SAF 94-310, SAF 95-1577) and Junta de Castilla y León (SA08/93, SA23/94) for financial support of this work.

References and Notes

1. San Feliciano, A.; Caballero, E.; Pereira, J. A. P.; Puebla, P. *Tetrahedron* **1991**, *47*, 6503.
2. Caballero, E.; Puebla, P.; Medarde, M.; San Feliciano, A. *Tetrahedron* **1993**, *49*, 10079.
3. Caballero, E.; Puebla, P.; Medarde, M.; Honores, Z.; Sastre, P.; López, J. L.; San Feliciano, A. *Tetrahedron Lett*. **1998**, *39*, 455.
4. San Feliciano, A.; Caballero, E.; Puebla, P.; Pereira, J. A. P.; Gras, J.; Valenti, C. *Eur. J. Med. Chem*. **1992**, *27*, 527.
5. Morán, A.; Martín, E.; Velasco, C.; Martín, M. L.; San Román, L.; Caballero, E.; Puebla, P.; Medarde, M.; San Feliciano, A. *J. Pharm. Pharmacol*. **1997**, *49*, 421.
6. Bayer, R.; Kalusche, D.; Kaufmann, R.; Mannhold, R. *Naunyn-Schmiedeb. Arch. Pharmacol*. **1975**, *290*, 81.
7. Sato, M.; Nagao, T.; Yamaguchi, I.; Nakajima, H.; Kiyomoto, A. *Arzneim. Forsch. Drug Res*. **1971**, *21*, 1338.
8. Towart, R.; Wehinger, E.; Meyer, H. *Naunyn-Schmiedeb. Arch. Pharmacol*. **1981**, *317*, 183.
9. Okumura, K.; Ichihara, K.; Nagasaka, M.; Oda, N.; Tajima, K. *Eur. J. Pharmacol*. **1993**, *235*, 69.
10. Mikus, G.; Mast, V.; Ratge, D.; Wisser, H.; Eichelbaum, M. *Clin. Pharmacol. Ther*. **1995**, *57*, 52.
11. Tamazawa, K.; Arima, H.; Kojima, T.; Isomura, Y.; Okada, M.; Fujita, S.; Furuya, T.; Takenaka, T.; Inagaki, O.; Terai, M. *J. Med. Chem*. **1986**, *29*, 2504.
12. Goldmann, S.; Stoltefuss, J. *Angew. Chem., Int. Ed. Engl*. **1991**, *30*, 1559.
13. Goldmann, S.; Stoltefuss, J.; Born, L. *J. Med. Chem*. **1992**, *35*, 3341.
14. Ashimori, A.; Uchida, T.; Ohtaki, Y.; Tanaka, M.; Ohe, K.; Fukaya, C.; Watanabe, M.; Kagitani, M.; Yokoyama, K. *Chem. Pharm. Bull*. **1991**, *39*, 108.
15. Enders, D.; Müller, S.; Demir, A. S. *Tetrahedron Lett*. **1988**, *29*, 6437.
16. Ebiike, H.; Maruyama, K.; Achiwa, K. *Tetrahedron: Asymmetry* **1992**, *3*, 1153.
17. Ikeda, K.; Kato, T.; Suzuki, T.; Achiwa, K. *Chem. Pharm. Bull*. **1998**, *46*, 518.
18. Puebla, P., Gotor, V., unpublished results.
19. Caballero, E.; Puebla, P.; Medarde, M.; Sánchez, M.; Salvadó, M. A.; García-Grande, S.; San Feliciano, A. *J. Org. Chem*. **1996**, *61*, 1890.
20. Caballero, E.; Puebla, P.; Sánchez, M.; Medarde, M.; Morán, L.; San Feliciano, A. *Tetrahedron: Asymmetry* **1996**, *7*, 1985.
21. Data for **5r**: Yellow solid, mp 99–100 °C (hexane:ether); $[\alpha]_D$ +13.5 (c = 0.50, $CHCl_3$); IR (film): 1734, 1682, 1567 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.10 (s, H-2′), 8.04 (d, J = 8, H-4′), 7.61 (d, J = 8, H-6′), 7.44 (t, J = 8, H-5′), 4.37 (s, H-7), 4.0-4.3 (m, H-2, H-3a,-C**H$_2$**–CH_3), 3.45 (s, H-13), 3.29 (s, H-8), 3.20 (m, H-3b), 2.57 (s, H-9), 1.32 (t, J = 6.2, -CH_2-C**H$_3$**), 1.28 (d, J = 5.7, C**H$_3$**-C_2), 0.84 (s, H-12); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 171.0 (C-11), 169.0 (C-10), 152.8 (C-5), 148.4 (C-3′), 147.7 (C-1′), 133.9 (C-6′), 129.1 (C-5′), 122.6 (C-2′), 121.3 (C-4′), 91.3 (C-6), 90.1 (C-8a), 72.9 (C-2), 60.7 (-CH_2–CH_3), 53.1 (C-8), 52.2 (C-3), 50.6 (C-13), 40.9 (C-7), 28.7 (C-12), 20.3 (**C**H_3–C_2), 18.9 (C-9), 14.3 (-CH_2–**C**H_3). Data for **5s**: $[\alpha]_D$ −15.2 (c = 0.55, $CHCl_3$).
22. Smith, D. F. *Pharmacol. Toxicol*. **1989**, *65*, 321.
23. Eltze, M.; Boer, R.; Sanders, K. H.; Boss, H.; Ulrich, W. R.; Flockerzi, D. *Chirality* **1990**, *2*, 233.
24. Hof, R. P.; Rüegg, U. T.; Hof, A.; Vogel, A. *J. Cardiovasc. Pharmacol*. **1985**, *7*, 689.
25. Niva, T.; Tokuma, Y.; Nakagawa, K.; Nogushi, H.; Yamazoe, Y.; Kato, R. *Res. Commum Chem. Pathol. Pharmacol*. **1988**, *60*, 161.