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Derivatization of (±)-5-[(2-Methylphenoxy)methyl]-2-amino-2-oxazoline, an Imidazoline Binding Sites Ligand, with (+)-(*R*)-α-Methylbenzyl Isocyanate for Drug Monitoring Purposes

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The derivatization of racemic 5-[(2-methylphenoxy)-methyl]-2-amino-2-oxazoline, developed as an imidazoline binding sites ligand, with (+)-(*R*)-α-methylbenzyl isocyanate was performed in chloroform. The reaction led to two pairs of diastereomers, which were separated by RP-HPLC. A kinetic study of the derivatization reaction was achieved in order to establish conditions suitable for experimental drug monitoring.

Keywords: HPLC; 2-amino-2-oxazoline; Derivatization; Enantioseparation; Pharmacokinetics; Imidazoline ligand

INTRODUCTION

For several years, we mainly focused our attention on the chemistry of 2-amino-2-oxazolines developed either as synthons or as potentially active compounds.[1–3] Recently, we designed 5-[(2-methylphenoxy)methyl]-2-amino-2-oxazoline **1** (S22687), a bioisosteric compound of rilmenidine, developed as an imidazoline receptor selective ligand.[4,5] Recent research suggests the involvement of the imidazoline binding sites in mood disorders such as major depression.[6–9] The stereospecificity of the imidazoline binding sites has been investigated, leading us to synthesize the (+)-(*S*) (S23229) and (−)-(*R*) (S23230) enantiomers of **1**. The affinity for I_1 sites was determined on bovine adrenals using [^{3}H]clonidine; both compounds bind to I_1 sites with high affinity, the K_i being 3.7×10^{-7} M and 5.8×10^{-9} M, respectively. The affinity for I_2 sites was determined on rabbit renal cortex using [^{3}H]idazoxan; both compounds bind to I_2 sites with high affinity, the K_i being 4.5×10^{-8} M and 1.1×10^{-8} M, respectively. For pharmacokinetic purposes we investigated the HPLC enantioseparation of racemic 5-[(2-(methylphenoxy)-methyl]-2-amino-2-oxazoline (*rac*-**1**) after derivatization with (+)-(*R*)-α-methylbenzyl isocyanate **2**.

The condensation of isocyanates with amino derivatives constitutes a simple and practical route for the preparation of diversely functionalized ureas.[10] Moreover, chiral isocyanates are often used as chiral derivatizing agents for the indirect resolution of amines by chromatographic separation of diastereomeric derivatives.[11–13] Applied to 2-amino-2-oxazolines the reaction of isocyanate can lead to complex mixtures linked to a specific structural unit. The amidine group of 2-amino-2-oxazolines induces a tautomeric phenomenon with two limit forms. The exocyclic and endocyclic nitrogen atoms are potent nucleophilic centers. Consequently, two regioisomers are obtained through the reaction of an isocyanate. They differ in the position of the corresponding carbamoyl moiety, substituted either on the endocyclic or the exocyclic nitrogen atom. Finally, the derivatization of a racemic 2-amino-2-oxazoline with (+)-(*R*)-**2** could lead to a mixture of four compounds, *i.e.* each of the regioisomers giving a mixture of two diastereomers.

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In the analytical part of this work, we report the study of the derivatization reaction of the racemic 5-[(2-methylphenoxy)methyl]-2-amino-2-oxazoline (*rac*-**1**) with (+)-(*R*)- α-methylbenzyl isocyanate **2**. The enantioseparation of *rac*-**1** was performed by using a RP-HPLC procedure. Moreover, the derivatization reaction rate was investigated by working at two temperatures. The effects of some reaction parameters were studied, partly based on the chromatographic results. The work begins with a synthesis part. It reports the chemical procedure established for the synthesis of pure diastereomers used as reference compounds for identification of the chromatographic peaks. Structural assignment for all the new compounds is discussed, and is mainly based on spectroscopic data.

MATERIALS AND METHODS

Chemistry

Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and are reported uncorrected. Infrared (IR) spectra were determined in KBr discs on a BRUKER IFS-25 spectrometer. NMR spectra were recorded on a BRUKER AC 200 spectrometer (200 MHz). Chemical shifts refer to tetramethylsilane which was used as an internal reference. OH appeared as a singlet exchangeable with D_2O. Elemental analyses were conducted by CNRS, Vernaison, France and the results were within ±0.3% of their calculated values.

Reagents and Chemicals

HPLC-grade acetonitrile and methanol were purchased from Acros (Geel, Belgium) and Baker (Deventer, Holland), respectively. Diethyl ether was obtained from Cooper (Melun, France) and was of analytical-grade. The (+)-(*R*) and (−)-(*S*) enantiomers and the racemic form of 5-[(2-methylphenoxy) methyl]-2-amino-2-oxazoline **1** were previously synthesized by the authors.[4] (+)-(*R*)-α-Methylbenzyl isocyanate **2** (enantiomeric excess ≥99.0%) was furnished by Sigma-Aldrich (St. Quentin Fallavier, France).

Reaction of (R)- or (S)-5-[(2-methylphenoxy)-methyl]-2-amino-2-oxazoline (1) with (+)-(R)-α-methylbenzyl Isocyanate (2)

This reaction, based on a procedure already described,[14] was used for the preparation of pure (*R*)-5- and (*S*)-5-[(2-methylphenoxy)methyl]-3-[(*R*)-α-methylbenzylcarbamoyl]-2-iminooxazolidines **3** and **4**. To a solution of 0.1 mole of (*R*)- or (*S*)-5-[(2-methylphenoxy)methyl]-2-amino-2-oxazoline **1** in ethanol (200 mL) at 20°C was added 0.1 mole of (+)-(*R*)-α-methylbenzyl isocyanate **2** in ethanol (20 mL). The mixture was stirred at 20°C for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel using chloroform–methanol (90:10) as eluent. From the single enantiomer (*R*)-**1** the two (*R*)–(*R*)-**3** (59%) and (*R*)–(*R*)-**5** (9%) were isolated after purification (ratio 87/13), and from the single enantiomer (*S*)-**1** we obtained (*S*)–(*R*)-**4** (66%) and (*S*)–(*R*)-**6** (11%) (ratio 86/14).

(*R*)-5-[(2-methylphenoxy)methyl]-3-[(*R*)-α-methylbenzylcarbamoyl]-2-iminooxazolidine, **3**

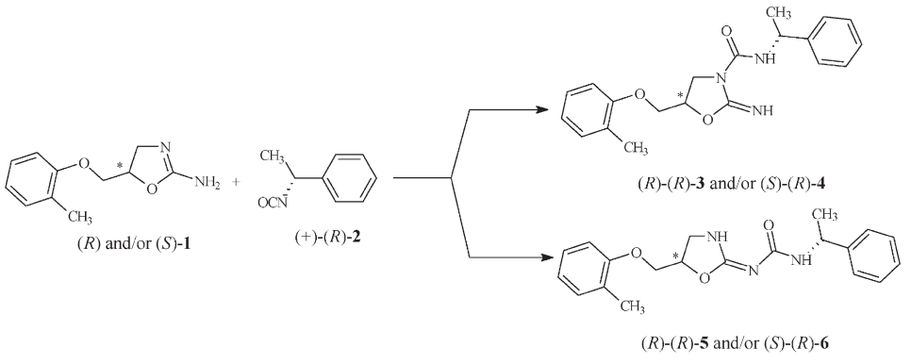
As pale yellow oil (59% yield). IR: 3355, 3150 (NH), 1690 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 1.52 (3H, d, *J* 7.0, CHC*H*$_3$), 2.20 (3H, s, *C*H$_3$), 4.10–4.14 (4H, m, H-4 and CH_2O), 4.78 (1H, m, H-5), 5.05 (1H, qt, *J* 7.6, C*H*CH$_3$), 5.86 (1H, s, NH), 6.76 (1H, d, *J* 7.80 H-3′), 6.90 (1H, t, *J* 7.80 H-4′), 7.13 (2H, m, H-2″ and H-6″), 7.16 (2H, m, H-5′ and H-6′), 7.33 (3H, m, H-3″, H-5″, H-4″), 9.71 (1H, d, *J* 7.60, NH); ^{13}C-NMR ($CDCl_3$) δ 16.0 (CH_3), 23.1 (CH_3), 45.8 (C-4), 49.9 (CH), 68.2 (CH_2O), 73.1 (C-5), 110.8 (C-Ar), 121.3 (C-Ar), 126.0 (2C-Ar), 126.8 (C-Ar), 127.0 (C-Ar), 128.6 (2C-Ar), 130.9 (C-Ar), 144.0 (C-Ar), 152.0 (C = N), 156.0 (CO). Elemental analysis ($C_{20}H_{23}N_3O_3$) C, H, N.

(*S*)-5-[(2-methylphenoxy)methyl]-3-[(*R*)-α-methylbenzylcarbamoyl]-2-iminooxazolidine, **4**

As white crystals (66% yield), mp 94°C (diethyl ether). IR: 3355, 3210 (NH), 1700 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 1.33 (3H, d, *J* 6.90, CHC*H*$_3$), 2.10 (3H, s, *C*H$_3$), 3.99–4.19 (4H, m, H-4 and CH_2O), 4.80 (1H, m, H-5), 5.04 (1H, qt, *J* 7.6, C*H*CH$_3$), 5.87 (1H, s, NH), 6.71 (1H, d, *J* 7.85, H-3′), 6.90 (1H, t, *J* 7.85, H-4′), 7.12 (2H, d, *J* 7.4, H-2″ and H-6″), 7.23 (2H, m, H-5′ and H-6′), 7.31 (3H, m, H-3″, H-4″, H-5″), 9.72 (1H, d, *J* 7.60, NH); ^{13}C-NMR ($CDCl_3$) δ 16.0 (CH_3), 23.1 (CH_3), 45.8 (C-4), 49.9 (CH), 68.2 (CH_2O), 73.0 (C-5), 110.7 (C-Ar), 121.3 (C-Ar), 126.0 (2C-Ar), 126.8 (C-Ar), 127.0 (C-Ar), 128.5 (2C-Ar), 130.9 (C-Ar), 144.0 (C-Ar), 152.0 (C = N), 156.0 (CO). Elemental analysis ($C_{20}H_{23}N_3O_3$) C, H, N.

(*R*)-5-[(2-methylphenoxy)methyl]-2-[(*R*)-α-methylbenzylcarbamoyl]-2-iminooxazolidine, **5**

As white crystals (9% yield), mp 140°C (ethanol). IR: 3345 (NH), 1650 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 1.44 (3H, d, *J* 6.80, CHC*H*$_3$), 2.17 (3H, s, CH_3), 3.74 (2H, m, H-4), 4.10 (2H, d, *J* 4.10, OCH_2), 4.93 (2H, m, H-5, C*H*CH$_3$), 5.75 (1H, bs, NH), 6.74 (1H, d, *J* 7.70, H-3′), 6.87 (1H, t, *J* 7.70, H-4′), 7.11 (2H, d, *J* 7.05, H-2″ and H-6″), 7.21 (2H, m, H-5′ and H-6′), 7.29 (3H, m, H-3″, H-4″ and H-5″), 8.66 (1H, bs, NH); ^{13}C-NMR ($CDCl_3$) δ 16.0 (CH_3), 22.8 (CH_3), 44.5(C-4), 67.7 (CH_2O), 74.4 (C-5), 110.9 (C-Ar), 121.3 (C-Ar), 125.8 (2C-Ar), 126.8 (2C-Ar), 128.5 (2C-Ar), 130.9 (C-Ar), 144.4 (C-Ar), 156.1 (C = N), 157.3 (CO). Elemental analysis ($C_{20}H_{23}N_3O_3$) C, H, N.



SCHEME 1 Synthetic pathway of the reaction between **1** and (+)-(R)-**2**.

(*S*)-5-[(2-methylphenoxy)methyl]-2-[(*R*)-α-methylbenzylcarbamoyl]-2-iminooxazolidine, **6**

As white crystals (11% yield), mp 123°C (diethyl ether). IR: 3335 (NH), 1645 cm^{-1} (CO), ^{1}H-NMR ($CDCl_3$) δ 1.44 (3H, d, *J* 6.7, CHCH_3), 2.14 (3H, s, CH_3), 3.72 (1H, m, H-4a), 3.85 (1H, m, H-4b), 4.09 (2H, d, *J* 4.30, OCH_2), 4.92 (2H, m, H-5, C*H*CH_3), 5.82 (1H, bs, NH), 6.73 (1H, d, *J* 7.70, H-3′), 6.87 (1H, t, *J* 7.70, H-4′), 7.10 (2H, d, *J* 7.15 H-2″ and H-6″), 7.20 (2H, m, H-5′ and H-6′), 7.28 (3H, m, H-3″, H-4″ and H-5″), 8.70 (1H, bs, NH); ^{13}C-NMR ($CDCl_3$) δ 16.0 (CH_3), 22.8 (CH_3), 44.6 (C-4), 49.7 (CH), 67.8 (CH_2O), 74.4 (C-5), 110.9 (C-Ar), 121.3 (C-Ar), 125.7 (2C-Ar), 126.8 (2C-Ar), 128.5 (2C-Ar), 130.9 (C-Ar), 144.2 (C-Ar), 156.1 (C = N), 157.2 (CO). Elemental analysis ($C_{20}H_{23}N_3O_3$) C, H, N.

Study of the Derivatization Reaction Rate Between Rac-1 and 2

Instrumentation and Chromatographic Conditions

The chromatographic apparatus (Waters, Milford, MA, USA) was equipped with a constant flow pump M 501, a Model 2487 spectrophotometer detector and a Model 746 integrator-recorder.

The separation was performed on a Supelcosil™ ABZ + Plus analytical column (250 × 4.6 mm I.D.; 5 μm particle size) from Sigma-Aldrich (St. Quentin Fallavier, France) at 0°C or +20°C. The mobile phase consisted of M/15 phosphate buffer (pH 7), methanol and acetonitrile (45:30:25, v/v/v). The mobile phase was filtered (0.5 μm) and degassed immediately prior to use and an in-liner 2 μm filter (Waters-Assoc.) was positioned ahead of the column. The flow rate was 1.2 ml/min. The compounds were chromatographed, using 254 nm for detection, within 25 min.

Derivatization

The derivatization reaction was performed at 0 and 20°C with equimolar amount (0.1 mmol) of *rac*-**1** and **2** dissolved in chloroform (1 mL). Aliquots, collected at different times, were diluted in the mobile phase (composition described below) and stocked at − 20°C until analysis. The quantification of all compounds was achieved by using the described chromatographic method. The percentage of obtained products %(X) was calculated according to: %(X) = [peak area of product X/total amount of peak areas of chromatographed products)] × 100. The plot of X against time was used for monitoring the progress of the reaction.

RESULTS AND DISCUSSION

Chemistry

The synthesis of new compounds **3**, **4** and **5**, **6** was achieved for identification purposes according to a procedure perfected by the authors (Scheme 1).[14] The reaction of pure enantiomer (*R*)-**1** with (+)-(*R*)-α-methylbenzyl isocyanate **2** in ethanol at 20°C during 1 hour gave a mixture of regioisomers (*R*)–(*R*)-**3** (59%) and (*R*)–(*R*)-**5** (9%) after purification on a silica gel column. On the other hand, the reaction of (*S*)-**1** with (+)-(*R*)-α-**2** gave regioisomers (*S*)–(*R*)-**4** (66%) and (*S*)–(*R*)-**6** (11%).

All compounds have different physicochemical properties, as illustrated by the differences in melting points, *i.e.* 94°C for (*S*)–(*R*)-**4**, 140°C for (*R*)–(*R*)-**5** and 123°C for (*S*)–(*R*)-**6**, while (*R*)–(*R*)-**3** was isolated as an oil.

The main spectroscopic difference between the regioisomers was observed in the ^{1}H-NMR spectra. For **3** and **4**, the chemical shift of the *NH*CH function is observed at 9.71 or 9.72 ppm as a doublet (J = 7.60 Hz). It appeared at 8.66 or 8.70 ppm as a broad singlet for **5** and **6**, respectively. In the IR spectra, the C = O bonds were observed at 1690 or 1700 cm^{-1} for diastereomers **3** and **4**, and at about 1650 cm^{-1} for compounds **5** and **6**.

No significant difference was observed in the IR, ^{1}H- and ^{13}C-NMR spectra of diastereomers **3** and **4**.

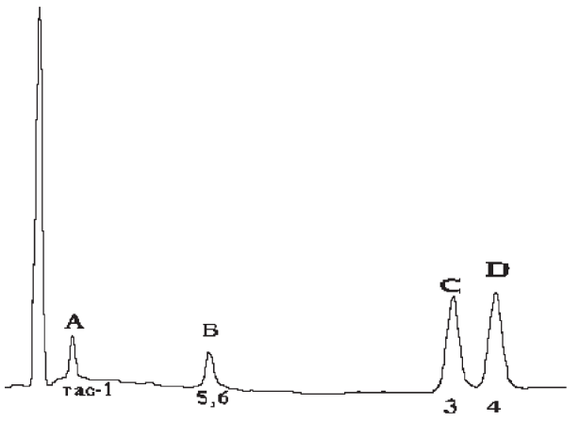


FIGURE 1 Chromatogram of diastereoisomers **5** and **6** (B), diastereoisomers **3** and **4** (C and D) obtained from derivatization of *rac*-**1**.

For the diastereomers **5** and **6**, only slight differences in ^{1}H-NMR were noticed, *i.e.*, the H-4 was observed at 3.74 ppm for **5** and at 3.72 and 3.85 ppm for **6**, probably due to a π interaction between the phenyl rings. The NH was present at 5.75 ppm for **5** and at 5.82 ppm for **6**.

Study of the Derivatization Reaction Between Rac-1 and (+)-(*R*)-α-methylbenzyl Isocyanate 2

For enantioseparation the derivatization reaction of *rac*-**1** with (+)-(R)-α-methylbenzyl isocyanate **2** was carried out at 20°C, as described in the experimental section. By using the selected eluent mobile phase, *i.e.* M/15 phosphate buffer (pH 7) methanol and acetonitrile (45:30:25, v/v/v), four peaks were observed in the chromatograph (Figure 1).

They were identified and assigned by comparison with the pure compounds *rac*-**1** and **3**–**6** (Table I).

The first peak (A) is due to the unreacted 2-amino-2-oxazoline *rac*-**1**. The second peak (B) was assigned to the diastereomers **5** and **6** having the same retention times. The two peaks (C) and (D) were assigned to the diastereomers **3** and **4**, respectively which exhibited identical areas (50/50). This equality suggested that the stereochemical conversion of the chiral reactant **2** was limited. Indeed, the risks involved in using chiral derivatizing agent for separation of enantiomers include racemisation of any stereogenic center during the derivatization and the limited enantiomeric purity of chiral derivatizing agent.[8]

TABLE I Retention times of derivatization products and *rac*-**1**

Compounds	*rac*-**1**	**3**	**4**	**5**	**6**
Retention time	4.9 min	19.8 min	21.0 min	9.3 min	9.3 min

For all compounds, the chromatographic profile can be understood in terms of polarity differences between species. They are linked to the tendency of the most basic exocyclic nitrogen atom towards protonation, giving the corresponding imino protonated form (pKa of **1** = 8.23).[15] The ratio of both regioisomers, calculated from the integration of peaks B *versus* C and D, was 18/82, showing the predominance of regioisomers **3** and **4** versus **5** and **6**. This result agrees with the previously described differential chemical behaviour of the two nucleophilic nitrogen atoms of 2-amino-2-oxazolines.[14–16]

Study of the Derivatization Reaction Rate

According to previously reported theoretical results obtained for related amino heterocycles, the substitution of the endocyclic nitrogen atom should proceed with a smaller activation barrier than that of exocyclic nitrogen.[17] Moreover, it seemed that the addition of isocyanate should occur at the ring endocyclic nitrogen in a regiospecific manner to afford kinetic-favoured substitution. In the other hand, it was possible to consider an eventual transposition between the regioisomers, influenced by the temperature[14] so that it appeared important to investigate the kinetic parameters in relation to the final reaction composition.

In order to explore the rate of the reaction between *rac*-**1** and **2** we carried out the derivatization at 0°C and +20°C with equimolar amounts of reagents. The related percentage amounts of residual **1**, of regioisomers **3**–**4** and of regioisomers **5**–**6** were measured at known intervals of time using the previously described HPLC method. The plots of percentage amounts against time are depicted in Figure 2 (0°C).

The obtained curves have similar profiles at 20°C and at 0°C. In both cases the formation of the regioisomers **3**–**4** and **5**–**6** was simultaneous and the removal of **1** is complete after about 16 minutes. At both temperatures, **3**–**4** are the major regioisomers (about 84% of total reaction products). The effect of a temperature increase (20°C) appeared especially on the progress of derivatization within the first minute, but didn't affect the final percentage of reaction products. As illustrated by the quasi-linearity of all curves after 5 minutes, no transposition between the regioisomers **3**–**4** and **5**–**6** was noticed (Figure 2).

The progress of the reaction was studied through the consumption of the reactant **1**. For this purpose, it was assumed that the reaction at 0°C and 20°C between equimolar amounts of **1** and **2** follows a second-order rate law. The decrease in concentration of **1** was defined in terms of percentage amounts from the peak area measures. The slopes of the graph (k) were computed by using least squares linear regression analysis. At 20°C and 0°C, these lines

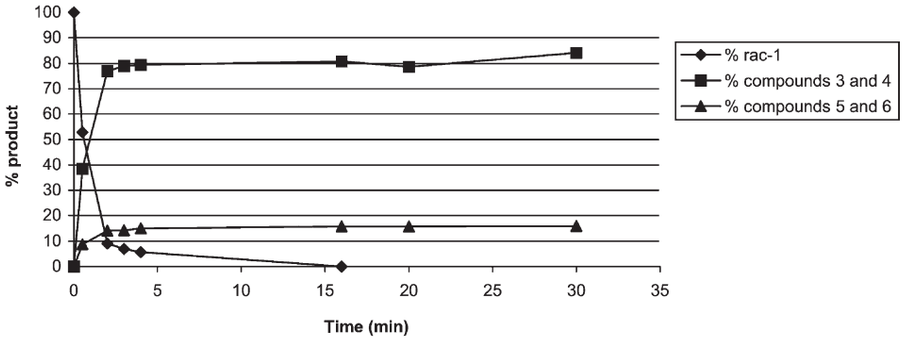


FIGURE 2 Reaction of *rac*-**1** with (+)-(*R*)-**2** and formation of four derivatization products **3**–**6** at 0°C ($n = 10$, number of assays per time).

exhibited excellent linearities, *i.e.* $r^2 = 0.98$ and 0.97 respectively, justifying the second-order rate law.

CONCLUSION

In this work, we investigated the derivatization reaction of the racemic 5-[(2-(methylphenoxy)methyl]-2-amino-2-oxazoline (S22687) **1**, an imidazoline binding sites ligand, with (+)-(*R*)-α-methylbenzyl isocyanate **2**. After derivatization, the enantioseparation of **1** was achieved through an HPLC method. Four related derivatization products were formed during the reaction. They were identified by comparison with synthesized reference compounds. The chromatographic analytical procedure was used for the study of the derivatization reaction rate law. The results will permit development of the enantioseparation of S22687, useful for the determination of its pharmacokinetic profile.

Acknowledgements

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