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Chromatographic resolution and pharmacological investigation of some imidazole derivatives

M.G. Quaglia[a,*], E. Bossù[a], G.C. Porretta[b], M. Biava[b], R. Fioravanti[b], L. Romanelli[c], A. Leonardi[d]

[a]*Dipartimento Studi Farmaceutici, Università degli Studi "La Sapienza", Piazzale A. Moro 5, 00185 Rome, Italy*
[b]*Dipartimento di Studi di Chimica e Tecnologie di Sostanze Biologicamente Attive, Università degli Studi "La Sapienza", P. le A. Moro 5, 00185 Rome, Italy*
[c]*Istituto di Farmacologia e Farmacognosia, Università degli Studi "La Sapienza", P. le A. Moro 5, 00185 Rome, Italy*
[d]*RD Division, Recordati SpA, Via Civitali 1, 20148 Milan, Italy*

Abstract

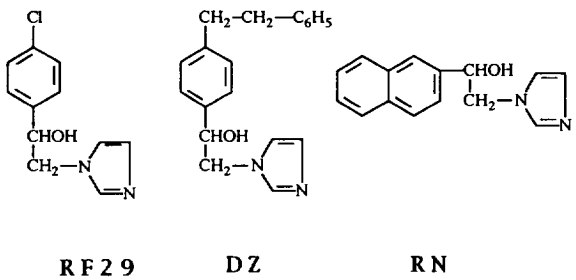
Some N-phenylethylimidazoles have a chiral carbon atom in their structure. These compounds were obtained by non-stereoselective synthesis and their pharmacological activity was always tested on the racemic mixtures. The proposed HPLC method allowed the chiral resolution of RF29, denzimol and reduced nafimidone by using a Chiracel OD-H column. The enantiomers of each compound were separately collected and their pharmacological activity was tested on the guinea pig ileum and the rabbit jejunum.

Keywords: Enantiomer separation; Phenylethylimidazoles; Imidazoles

1. Introduction

Some N-phenylethylimidazoles of pharmaceutical interest, containing an imidazole moiety in their structure, show antimicrobial, antifungal and anticonvulsivant activity, e.g., RF29,(4-chlorophenyl)-1*H*-imidazole-1-ethanol [1], or only anticonvulsivant activity e.g., denzimol (DZ), α-[4-(2-phenylethyl)phenyl]-1*H*-imidazole-1-ethanol) [2], and reduced nafimidone (RN), α-(2-naphthalenyl)-1*H*-imidazole-1-ethanol [3]. (For structures see Fig. 1.)

These compounds, all obtained by non-stereoselective synthesis, have a chiral carbon in their structure. The investigation of these molecules was previously carried out using the racemic mixture, and therefore the resolution of the racemate and the study of the chemical and



RF29 DZ RN

Fig. 1. Structures of RF29, DZ and RN.

* Corresponding author.

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pharmacological aspects of the separated enantiomers seemed of interest. In this work, the resolution of racemates was carried out using cellulose tris(3,5-dimethylphenylcarbamate) adsorbed on macroporous silica gel as a chiral stationary phase (CSP), obtaining a good separation of enantiomers. Then the enantiomers were separately collected and their absolute configuration and the correlation of stereoselectivity and biological activity were tested.

2. Experimental

2.1. Chemicals

All solvents and chemicals were of HPLC or analytical-reagent grade (Merck, Darmstadt, Germany). The chiral compounds were kindly supplied by the researchers who synthesized them.

2.2. Equipment

The chromatographic discrimination of all enantiomers was carried out with a Hewlett-Packard Series 1050 chromatograph equipped with an HP 1040 linear photodiode-array detector controlled by an HP 9000 Model 310 computer (Hewlett-Packard, Palo Alto, CA, USA). Sample solutions were injected via a Rheodyne Model 7125 valve using a 20-μl sample loop. The photodiode-array detector conditions were 225 and 270 nm, acquisition rate of spectra 1.280 ms, bandwidth for each channel 4, sensitivity range 50, reference wavelength 450 nm and reference bandwidth 50. Circular dichroism (CD) spectra were obtained with a Jasco 500-A dichograph, kindly provided by Professor P. De Santis (Dipartimento di Chimica, Università "La Sapienza", Rome, Italy).

2.3. Chromatographic analysis

The resolution of racemates was carried out by using commercial cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel as a chiral stationary phase (Chiracel OD-H, 5 μm; column 250 mm × 4.6 mm I.D., provided by Daicel Chemical Industries). The mobile phase was hexane–2-propanol with 0.15% (v/v) of diethylamine added. The hexane and 2-propanol and the conditions of separation were as follows: RF29, 70:30, containing 0.15% of diethylamine, at a flow-rate of 0.6 ml/min; DZ, 60:40, containing 0.15% of diethylamine, at a flow-rate of 0.6 ml/min; and RN, 30:70, containing 0.15% of diethylamine, at a flow-rate of 0.4 ml/min.

The identity in the UV spectra of each pair of enantiomers was checked and they were separately collected in several runs. The total amount of enantiomer collected was about 250 μg. The optical purity of the separated enantiomers was determined. The mobile phase solutions were evaporated under vacuum and the residues dissolved in ethanol for further tests (circular dichroism and biological assay). The concentration of ethanolic solutions was determined by RP-HPLC using calibration graphs obtained with the racemates.

2.4. Circular dichroism spectra

CD spectra were recorded on ethanolic solutions (4.2 m*M*) using 0.1-mm cells.

2.5. Pharmacological assay

The biological activity of the racemic mixtures of compounds RF29, DZ and RN and of the (−)- and (+)-enantiomers of DZ were tested in vitro following published methods [4,5]. The tests were carried out on two different tissues: (a) the guinea pig ileum, where the ability of RF29, DZ and RN to inhibit neuronal contractile activity of prostaglandin E1 was observed; and (b) the rabbit jejunum, by measuring the ability of N-phenylethylazole derivatives to inhibit the spontaneous activity of the tissue. This tissue was selected because of its multireceptorial activity, particularly for the presence of κ- and δ-opioid receptors. In each experiment the isolated tissue was set up under 1.5 g tension in a 10-ml organ bath containing tyrode solution at 37°C, bubbled with a gas mixture of O_2–CO_2 (95:5). Changes in tension were recorded by means of an isotonic

force transducer, allowing the preparation to equilibrate before the beginning of the experiment. The racemic mixture or separated enantiomers were solubilized in ethanol (1 mg/ml) and each solution was used to check their effect on the tissues by adding to the organ bath different concentrations in the range 50–5000 μg/l. In the guinea pig ileum assay, PGE_1 (10 ng) was added to the bath 1–2 min after addition of the compound under evaluation.

3. Results and discussion

Chiracel OD has been used successfully to resolve a wide variety of racemic drugs. The chiral recognition mechanism of this phase is well known [6–8] and a significant improvement in the resolution was obtained with Chiracel OD-H (5 μm). Baseline resolution of the racemic imidazole derivatives considered was obtained by adding to the mobile phase a small amount of diethylamine. The addition of a basic modifier to the eluent makes easier the exchange between adsorption and desorption of a basic solute on the stationary phase. The identities of two separated enantiomers were preliminarily confirmed by the perfect superimposition of their UV spectra. This method was successfully applied to the preparative separation of RF29 and reduced nafimidone enantiomers, which were separately collected. In contrast, the denzimol enantiomers were obtained chemically by esterefication with (+)-naproxen. The diastereomeric esters were separated chromatographically and then hydrolysed. The optical purities of all three pair of enantiomers were determined using the same chiral chromatographic column. The enantiomeric excesses (ee) for the first-eluted enantiomers and for the most retained enantiomer were, respectively, as follows: RF29, 98.3% and 94.86% ee; DZ, 98% and 95.2 ee; and RN, 99.4% and 97.79% ee.

The circular dichroism spectrum of each enantiomer was registered and compared with the spectra obtained analysing the (+)- and (−)-DZ enantiomers. Each pair of enantiomers showed the same strong bands in the 220–240-nm region and a second weak band in the 245–270-nm region, both of opposite sign. The strong band of the first-eluted enantiomers of RF29 and reduced nafimidone was positive. Also, (+)-denzimol showed a positive Cotton effect and, using the chiral HPLC method described above, it was eluted before the (−)-denzimol peak. This showed a CD spectrum having the same band as the (+)-enantiomer but with a negative Cotton effect. Further confirmation of the polarimetric sign and the elution order was obtained from RF29. The enantiomers of this compound were resolved also via diastereomeric salt formation using D-dibenzoyltartaric acid according to the method proposed by Godefroi and Herees [9]. On crystallization only one diastereomer and then the free enantiomer were obtained. This enantiomer, which has a positive Cotton effect, elutes first in chiral HPLC.

All compounds, especially denzimol, inhibit the neuronal contractile activity of prostaglandin PGE_1. No differences in the intensity of biological activity among the isolated enantiomers and racemic mixtures were found. In fact, the contractile activities of PGE_1 on the tissue, pretreated with a single enantiomer or racemic mixtures of RF29, DZ or RN, decreased equally. The rabbit jejunum was selected because of its multireceptorial activity and particularly for the presence of κ- and δ-opioid receptors. The common opioid agonists strongly depress the spontaneous activity of the tissue and, moreover some of the agonists strongly depress the spontaneous activity of the tissue and, moreover some of the agonists have shown anticonvulsant activity in various models of epilepsy. The three drugs showed the ability to relax this tissue and among them denzimol was the most active. Both denzimol enantiomers showed a similar behaviour, but with different intensity: the (+)-enantiomer was much more active than the (−)-enantiomer. The action of denzimol persisted also in the presence of the usual opioid (κ, δ), adrenergic, cholinergic and the GABA antagonists.

The separated enantiomers of RF29 and reduced nafimidone were equally active, but a higher concentration (5 μg/ml) was necessary.

Their activity was inhibited by atropine, whereas propanolol inhibited only RF29.

The data obtained in this research do not allow us to assign the absolute configuration to RF29, DZ and RN, but looking at a compound, such as econazole, structurally related to the tested compounds, a hypothesis can be formulated. A study of the absolute configuration of econazole [10], an imidazole derivative with antifungal activity, indicated that its levorotatory isomer has the *R* configuration. (−)-Econazole was prepared by etherification of the corresponding (−)-3-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol without formal inversion, but without racemization. Hence this intermediate can be considered to have the *S* configuration and its (+)-isomer the *R* configuration. From the results reported in Ref. [9], where (+)-α-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol was prepared by the same synthetic route as used in the synthesis of (+)-RF29, we conclude that (+)-RF29 could have the *R* configuration, which could also be extended to (+)-DZ and (+)-RN.

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