



A convenient synthesis by microwave irradiation of an active metabolite (EXP-3174) of losartan

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Abstract—A novel and convenient microwave-assisted synthesis of an active metabolite (EXP-3174) of losartan is described. Room temperature and microwave irradiation of the reactions are compared. Synthesis by microwave irradiation gave the desired compound in higher yields and in shorter reaction times than those obtained at room temperature. © 2003 Elsevier Science Ltd. All rights reserved.

Several innovative targets for antihypertensive new drugs discovery have been explored and novel molecules identified. The compounds are in various stages of development and commercialisation. Attempts to develop therapeutically useful angiotensin II receptor antagonists date to the early 1970s, and these initial endeavors concentrated on angiotensin peptide analogs.¹ Saralasin, 1-sarcosine, 8-isoleucine angiotensin II, and other 8-substituted angiotensins were potent angiotensin II receptor antagonists but were of no clinical value because of lack of oral bioavailability and because all peptide angiotensin receptor antagonists expressed unacceptable partial agonist activity.

Although initial efforts to develop nonpeptide angiotensin receptor antagonists were unsuccessful, an exciting breakthrough came in the early 1980s with the issuance of patents² on a series of imidazole-5-acetic acid derivatives that attenuated pressor responses to angiotensin II in rats. Two of the compounds described in the patents, S-8307 and S-8308, later were found to be highly specific, albeit very weak, nonpeptide angiotensin II receptor antagonists that were devoid of partial agonist activity.³ In an instructive example of drug design, molecular modelling of these lead com-

pounds gave rise to the hypothesis that their structures would have to be extended to mimic more closely the pharmacophore of angiotensin II. Through a clever series of stepwise modifications, the losartan (DuP 753) appears to have the greatest potential for the chronic oral treatment of essential hypertension.⁴ Losartan was approved by the United States Food and drug administration in 1995 for clinical use. Losartan has an oral bioavailability of about 33% and is rapidly absorbed (peak plasma levels in 1 h). Losartan itself is a potent, competitive AT₁ receptor antagonist; however, it has a short-life (about 2 h). The effectiveness of a once-a-day dosing regimen is explained by the fact that a discrete percentage of an oral dose of losartan in human beings is converted to a 5-carboxylic acid metabolite, designated EXP-3174,⁵ which is a non-competitive AT₁ receptor antagonist that is 10–40 times more potent than losartan and has a much longer half-life (6–9 h) than losartan.

Knowing the importance of the EXP-3174 in the mechanism of action of the losartan and considering that the literature on this subject, although very copious, is mainly constituted by patents, our attention has turned to improve the synthesis of this important metabolite bearing in mind new synthetic methodologies. The application of microwave energy to organic compounds for conducting synthetic reactions at highly accelerated rates is an emerging technique.^{6,7} In fact in later years, microwaves have become popular among synthetic organic chemists both to improve classical organic reac-

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tions, shortening reaction times and/or improving yields, as well as to promote new reactions.

These observations led us to check into the possibility for improvement in methods used for the synthesis of the EXP-3174. Moreover EXP-3174 could be a useful 'lead compound' for rational design of new drugs.

This paper describes a facile synthesis of EXP-3174 by application of microwave energy in the presence of solvents. Conventional heating (oil bath) and microwave irradiation of the reactions are compared.

The synthetic procedure,^{4a} summarized in Table 1, was performed according to that reported in literature⁸ and using a microwave oven (ETHOS 1600, Milestone) especially designed for organic synthesis. The experimental conditions used in our work were similar, with the same concentration of starting material and volume of solvent, to those used by conventional heating.

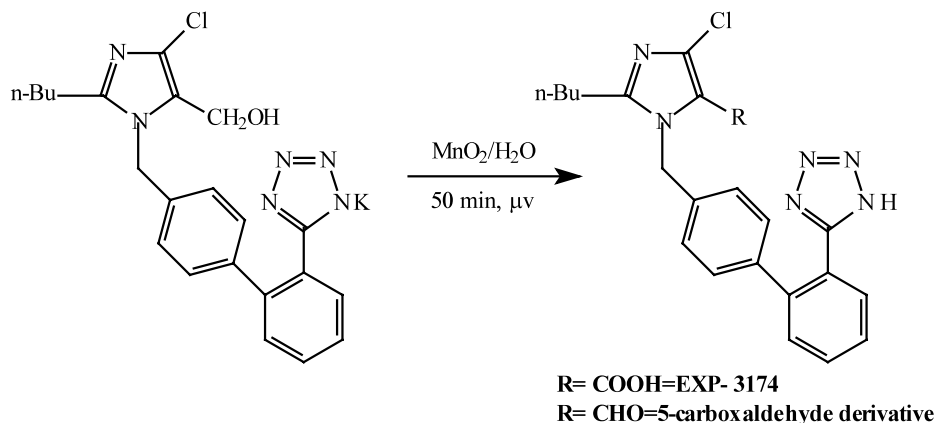
All reactions were performed in standard Pyrex glassware with a reflux condenser fitted through the roof of the microwave cavity and were performed by microwave program which was composed by appropriate ramping and holding steps. Identification of the optimum profile power/time and temperature for the synthesis was reported in Table 1. The temperature of the stirred reaction mixture was monitored directly by a

microwave-transparent fluoroptic probe inserted into the solution.

The general procedure reported in Table 1 is as follows:⁸ EXP-3174 was prepared from losartan (acquired from Biosintetica Ltd) by oxidation with activated manganese dioxide in 20 ml of H₂O by application of microwave energy for 50 min.⁸ The desired parameters (microwave power, temperature and time) were set as reported in Table 1. The reaction was monitored by TLC. After irradiation, the solution was treated as follows. The excess of MnO₂ was filtered, the solvent was removed and the crude residue was purified by RP-HPLC preparative obtaining 1.0 g (2.28 mmol) of EXP-3174 and 0.253 mmol of the corresponding aldehyde.⁸ EXP-3174 and the corresponding 5-carboxaldehyde derivative were characterized by ¹H NMR and by MS and the data were consistent with the considered structures.^{8,9}

The main advantage to this synthetic route is that a short time of irradiation of the mixture reaction provided the imidazole-5-carboxaldehyde as the major product. In fact a time of irradiation of 15 min furnished a 8:2 ratio of the imidazole-5-carboxaldehyde derivative.⁹ In contrast a prolonged irradiation (50 min) furnished a 9:1 ratio of EXP-3174. If the application of microwave energy was performed for longer time (>50 min) the losartan was not completely transformed in the corresponding acid derivative.

Table 1. Conventional heating versus microwave irradiation of EXP3174^a



| Compd. | Conventional heating ^b | | | Microwave irradiation | | | |
|---------------------------------------|-----------------------------------|----------|------------|------------------------|------------|--------------|------------|
| | Yield ^c (%) | Time (h) | Temp. (°C) | Yield ^c (%) | Time (min) | Power (Watt) | Temp. (°C) |
| EXP 3174 | 9 | 100 | Reflux | 64 | 2 | 200 | 80 |
| | | | | | 3 | 250 | 120 |
| | | | | | 5 | 300 | 160 |
| | | | | | 40 | 350 | 180 |
| Imidazole-5-carboxaldehyde derivative | 30 | 100 | Reflux | 60 | 1 | 200 | 80 |
| | | | | | 2 | 250 | 120 |
| | | | | | 3 | 300 | 160 |
| | | | | | 9 | 350 | 180 |

^a All the reactions were performed 3 times and the reaction time and yields given are the average values.

^b Oil-bath.

^c With respect to the starting amount of Losartan.

The aldehyde and the corresponding acid derivative can be utilized as intermediates in the preparation of a variety of other analogues. The aldehyde derivative and the EXP-3174 were tested on rabbit aorta preparation to evaluate the angiotensin II induced contractions.¹⁰

The EC₅₀ obtained confirmed the pharmacological profile reported in literature (losartan: 0.019 μ M; aldehyde derivative: 0.02 μ M; EXP-3174: 0.001 μ M).^{11,12}

In conclusion, we have shown that the application of microwave irradiation improves the yields and significantly reduces reaction times in the solution synthesis of EXP-3174. This synthesis provide further example of the utility of microwave irradiation in organic synthesis in the presence of solvents.

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- Synthesis of EXP-3174*: A mixture of 1.47 g (3.5 mmol) of losartan and 2.87 g (33 mmol) of activated manganese dioxide in 20 ml of H₂O was inserted in standard Pyrex glassware with a reflux condenser fitted through the roof of the microwave cavity. The reaction was performed for 50 min. by microwave program which was composed by appropriate ramping and holding steps (Table 1). The reaction was monitored by TLC (CHCl₃/MeOH/CH₃COOH 95/5/0.5). After irradiation, the excess of MnO₂ was filtered, the solvent was removed under reduced pressure and the residue was purified by preparative RP-HPLC obtaining 1.0 g (2.28 mmol) of EXP-3174 (yield 64%). The homogeneity of the purified product was assessed by analytical RP-HPLC with a Vydac C18-column (5 μ m, 4.6×250 mm, spherical). Analytical determinations were carried out by two solvent systems: A: 10%, (v/v), acetonitrile in 0.1% TFA, B: 60% (v/v), acetonitrile in 0.1% TFA (linear gradient from 20% A to 80% B over 35 min, UV detection at 220 nm, flow rate 1 mL/min). Preparative RP-HPLC was performed by the same gradient used for the analytical determinations. The operational flow rate was 30 mL min⁻¹. The final compound was characterized by ¹H NMR and MS. NMR spectra was recorded on a Bruker WM 500 spectrometer using tetramethylsilane as an internal standard. Mp 176–178°C, ESI: 435 (MH⁺) ¹H NMR (DMSO-*d*₆): δ 7.69 (d, 1H, *J*=8 Hz), δ 7.66 (t, 1H, *J*=8 Hz), δ 7.59 (t, 1H, *J*=8 Hz), δ 7.53 (d, 1H, *J*=8 Hz), δ 7.08 (d, 2H, *J*=8.5 Hz), δ 6.97 (d, 2H, *J*=8.5 Hz), δ 5.60 (s, 2H), δ 2.58 (t, 2H, *J*=7 Hz), δ 1.53 (quint, 2H, *J*=7 Hz), δ 1.24 (sext, 2H, *J*=7 Hz), δ 0.82 (t, 3H, *J*=7 Hz).
- Synthesis of the imidazole-5-carboxaldehyde derivative*: The title compound was prepared from losartan by the procedure described for the preparation of EXP-3174. The reaction was performed for 15 min by microwave program which was composed by appropriate ramping and holding steps (Table 1). 0.883 g (2.10 mmol) of the imidazole-5-carboxaldehyde derivative (yield 60%). NMR spectra were recorded on a Bruker WM 500 spectrometer using tetramethylsilane as an internal standard. Mp 154–155°C, ESI: 419 (MH⁺) ¹H NMR (DMSO-*d*₆): δ 9.68 (s, 1H), δ 7.68 (d, 1H, *J*=8 Hz), δ 7.65 (t, 1H, *J*=8 Hz), δ 7.58 (t, 1H, *J*=8 Hz), δ 7.52 (d, 1H, *J*=8 Hz), δ 7.10 (d, 2H, *J*=8.5 Hz), δ 7.03 (d, 2H, *J*=8.5 Hz), δ 5.58 (s, 2H), δ 2.63 (t, 2H, *J*=7 Hz), δ 1.51 (quint, 2H, *J*=7 Hz), δ 1.26 (sext, 2H, *J*=7 Hz), δ 0.81 (t, 3H, *J*=7 Hz).
- Rabbit Aorta Preparation*: Male New Zealand white rabbits (2.0–2.5 kg, provided by CEMIB-UNICAMP) were anaesthetised with pentobarbital sodium (Hypnol®, 30–40 mg/kg, i.v.) and exsanguinated via the carotid artery. The thoracic aorta was removed and cleaned of adherent fat and connective tissue. Aortic rings (3–4 mm) were mounted in 10 ml organ baths containing Krebs–Henseleit solution NaCl, 118.4; KCl, 4.7; MgSO₄·7H₂O, 1.2; KH₂PO₄, 1.2; CaCl₂·2H₂O, 2.5; NaHCO₃, 25.0 and glucose, 11.1 mM, maintained at 37°C and bubbled with a mixture of 95% O₂ and 5% CO₂. Under a resting tension of 10 mN, isometric tension changes were recorded on a Power Lab 400™ data acquisition system (software Chart, version 4.0, AD Instruments, MA, USA), through force-displacement transducers (Ugo Basile, Varese, Italy). After equilibration for 1 h, a single contractile-response curve to the cumulative addition of angiotensin II (100 pM–30 nM) was constructed. The strips were then washed and allowed to relax to the baseline tension. Each strip was then incubated for 30

- min with a single concentration of compound (EXP-3174 or aldehyde derivative) before a second contractile-response curve to angiotensin II was obtained.
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