A MODIFIED SYNTHESIS OF TRITIUM LABELLED AMIDES FROM LABELLED CARBOXYLIC ACIDS AND AMINES USING EEDQ AS A REAGENT

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SUMMARY

The reaction conditions for the preparation of amides from labelled carboxylic acids and amines using EEDQ (2-ethoxy-1(2H)-quinolinecarboxylic acid, ethyl ester) as a reagent were modified for the synthesis of the tritium labelled benzamide Halopemide (N-{2-[4-(5-chloro-2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)-1-piperidinyl]ethyl}-4-fluorobenzamide), as EEDQ, reacted in an unexpected way, resulting in a 1:1 mixture of the benzamide and the N-carbethoxy derivative of the starting amine and of "unreacted" carboxylic acid.
The basicity of the starting amine influenced the yield of the benzamide. By optimalizing the reaction conditions, it was possible to convert the labelled benzoic acid quantitatively into the benzamide.

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INTRODUCTION

Halopemide (R 34301, N-{2-[4-(5-chloro-2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)-1-piperidinyl] ethyl} -4-fluorobenzamide), a new antipsychotic agent with interesting clinical properties was developed in a structure-activity relationship program by Soudijn and van Wijngaarden (1). In order to study the distribution and properties at the subcellular level a tritium-labelled product of high specific activity was required.

By catalytic dehalogenation of 2-chloro-4-fluorobenzoic acid with tritium gas, highly labelled 4-fluorobenzoic acid is readily available.

The usual route for the preparation of halopemide (scheme A), by conversion of the benzoic acid (I) to its halide, reaction of the halide with ethyleneimine and coupling with the piperidine derivate (II), could not be adapted for the miniscale synthesis due to complicated isolation and purification procedures. Moreover from an economical point of view, a one-step synthesis with a labelled product is generally preferable to a more-step synthesis.

EEDQ (2-ethoxy-1(2H)-quinolinecarboxylic acid, ethyl ester) is a convenient coupling reagent for the preparation of amides in high yields by reacting carboxylic acids and amines in a 1:1 molar ratio in tetrahydrofuran, benzene, methanol and other a-protic solvents, at room temperature during 1-12 hours (2).

Scheme B shows the reaction mechanism as suggested by Belleau. The carboxylic acid is activated by EEDQ(III) to form IV which results via a six membered transition state (IV arrows) in the mixed anhydride V and quinoline.

The "slow" formation of V and its rapid consumption (minimizing side reactions) and the readily removable byproducts (quinoline, carbon dioxide, and ethanol) would make EEDQ superior to the classical anhydride method. So EEDQ seems the coupling reagent of choice for the synthesis of the labelled halopemide.

2. METHODS AND MATERIALS

After a series of cold runs in order to investigate the optimal reaction conditions, it appeared that EEDQ reacted in an unusual way.

Following the procedure as described by Belleau by stirring equimolar parts of the benzoic acid (I) and of the amine VI with a 10 % molar excess of EEDQ (scheme C) in benzene and/or methanol at room temperature for several hours, hardly any benzamide was formed. At reflux temperature however, thin layer chromatography showed the disappearance of the starting amine, the formation of Halopemide and of an unknown product within two hours.

Quantitative thin layer chromatography showed that the yield of the benzamide was about 50 % of the theoretical.
By preparative thin layer chromatography the unknown product could be separated from halopemide and isolated. After purification its structure was established by ir, uv, mass spectrometry and elemental analysis.
It appeared to be the N-carbethoxy derivative of the starting amine. (ethyl{2-[4-(5-chloro-2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)-1-piperidinyl]ethyl} carbamate) (VIII). The structure of the product was proven by comparing it to the N-carbethoxyderivative prepared by a different unequivocal route of synthesis. This side reaction accounts for the low yield of Halopemide, as half the amount of amine VI is consumed by the mixed anhydride, to form the N-carbethoxy compound and half the amount of 4-fluoro benzoic acid is regenerated (scheme D). This means a loss of half of the labelled benzoic acid.

So the reaction was repeated with 1 part of benzoic acid, 2 parts of the amine and 2.2 parts EEDQ in methanol at reflux temperature.
After 2 hours unreacted amine was still present but quantitative thin-layer chromatography showed that only 25 % of the theoretical yield of Halopemide was obtained whereas 90 % of the N-carbethoxy derivative was formed, indicating the influence of the basicity of the starting amine on the course of the reaction.

To test this possibility the reaction was repeated with the mono-HBr salt of the amine VI.
After mixing 1 part of carboxylic acid with 2 parts of the HBr salt and 2.2 parts EEDQ in methanol and refluxing for 2 hours, thin layer chromatography showed that Halopemide was formed quantitatively together with a quantitative yield of the N-carbethoxy compound, justifying the surmise of the unusual way in which EEDQ reacted in the described circumstances. (table 1)

TABLE 1 (cf. Scheme D) Summary of the cold runs for the synthesis of Halopemide in different reaction conditions.

Molar ratio of the reactants			yield obtained and expressed as a percentage of the theoretical as suggested in Scheme D	
Benzoic acid	Amine	EEDQ	Halopemide (VII)	N-carbethoxy amine derivate (VIII)
1	1 (base)	1.1	50 %	40 %
1	2 (base)	2.2	25 %	90 %
1	1 (mono-HBr salt)	1.1	50 %	50 %
1	2 (mono-HBr salt)	2.2	100 %	100 %

3. RESULTS

3.1 Table 1 shows a summary of the cold runs for the synthesis of Halopemide (R 34301).

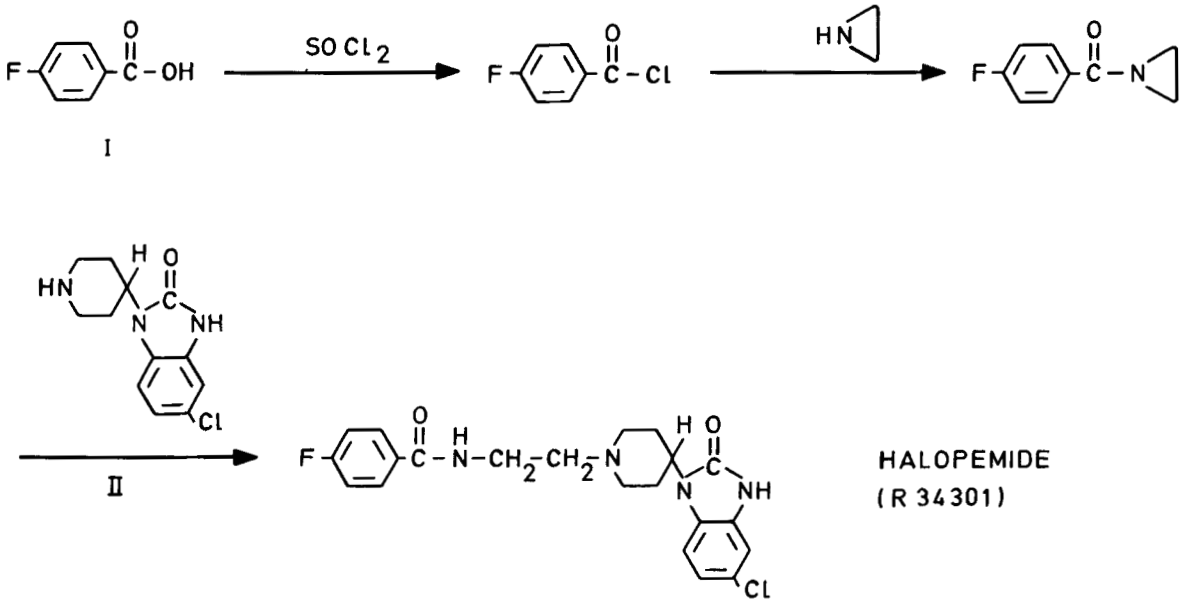
3.2 4-fluorobenzoic-2t-acid (I)

4 - fluorobenzoic - 2t - acid was labelled with tritium at I.R.E. (Fleurus, Belgium) by catalytic tritiation of 2-chloro-4 fluorobenzoic acid in dioxane and Pd/C 10 % as a catalyst and triethylamine as an HCl scavenger. The specific activity was 30 Ci/mM.
After dilution with unlabelled 4-fluorobenzoic acid to a specific activity of 10 Ci/mM the product was stored at -20°C in methanol at a concentration of 1 mCi per ml of methanol.

3.3 N-{2-[4-(5-chloro-2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)-1-piperidinyl] ethyl} -4-fluorobenzamide-2t (VII) = R 34301

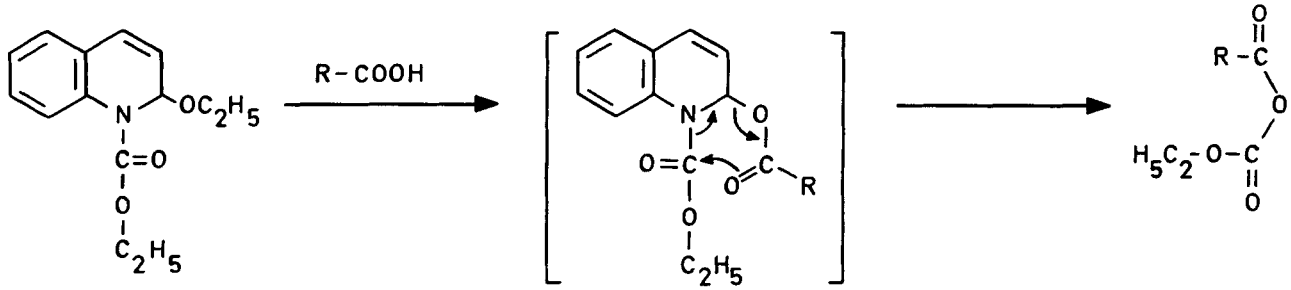
A 0.06 mMol aliquot of 4-fluorobenzoic-2t acid was evaporated to dryness in an oil-bath of 50° under a gentle current of dust-free ni-

SCHEME A



Scheme A. Usual route for the preparation of Halopemide
(N-{2-[4-(5-chloro-2, 3-dihydro-2-oxo-1H-benzimidazol-1-yl)-1-piperidinyl] ethyl} -4-fluorobenzamide)

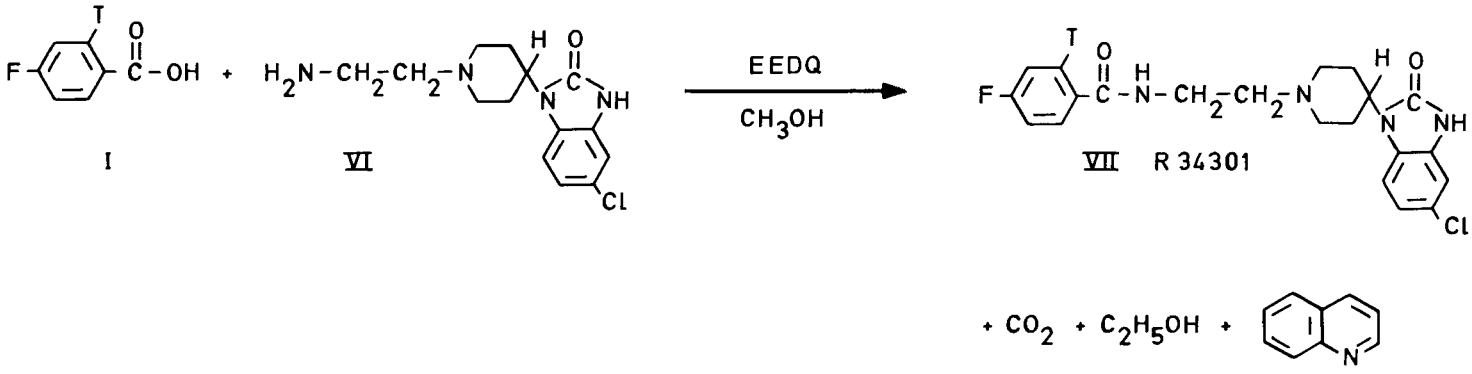
SCHEME B



III IV V

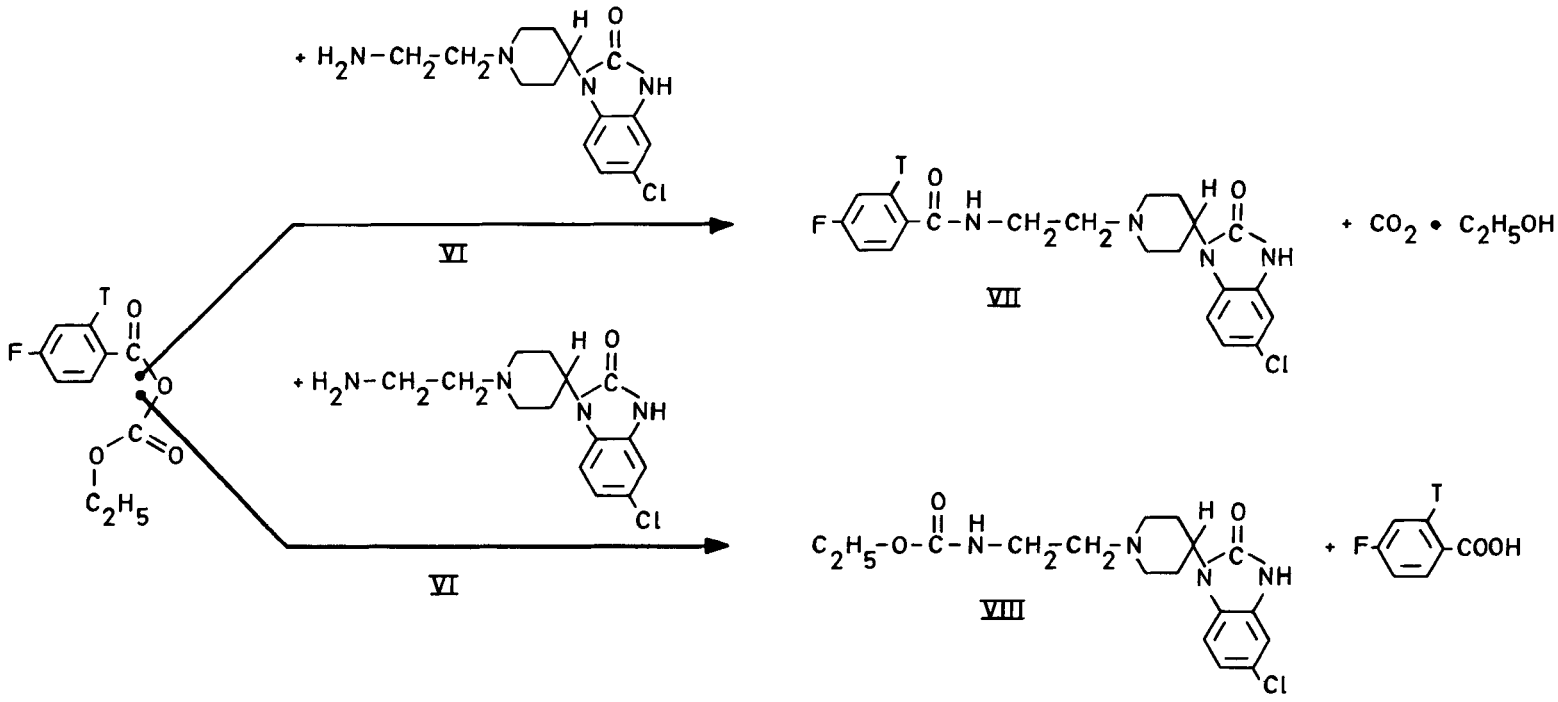
Scheme B. Reaction mechanism proposed by Belleau for the formation of the mixed anhydride from the reaction of EEDQ with carboxylic acids.

SCHEME C



Scheme C. Proposed route for the synthesis of tritium labelled Halopemide (see Belleau).

SCHEME D



Scheme D. Reaction scheme for the preparation of Halopemide, using the reaction conditions described by Belleau.

trogen and the residue dissolved in 0. 7 ml of methanol.
After adding 0. 12 mM 1-[1-(2-aminoethyl)-4-piperidinyl] -5-chloro-1, 3-dihydro-2 H-benzimidazol-2 one (VI) and 0. 13 mM EEDQ,the solution was heated in an oil-bath at 80 °C for 2. 5 hours, using a pear-shaped vessel, fitted with a reflux condenser.
After cooling, the reaction mixture was chromatographed on a preparative thin-layer plate, covered with a 0. 25 mm layer of Sil F 254 (Merck). The plate was developed with a mixture of 90 ml $CHCl_3$, 10 ml CH_3OH and 1 ml NH_4OH (conc.).

After location of ^{3}H-Halopemide by U. V. (254 nm) and scanning with a Berthold radio chromatograph scanner LB 2723, the zone was scraped off and extracted with methanol.

The solution was stored at -20 °C in a concentration of 1 mCi per ml.
The radiochemical purity was confirmed by thin-layer chromatography in three different solvent systems : $CHCl_3$ 90 + CH_3OH 10; $CHCl_3$ 85 + CH_3OH 15 + NH_4OH 1; acetate buffer solution (pH 4. 8) 5 + CH_3OH 18 + $CHCl_3$ 23 + ethylacetate 54 and by the inverse isotope dilution method.

The stability of the product was investigated at different pH by heating a small aliquot for 80 min in an oil bath of 60 °C in 0, 01 N HCl, 0, 1 N HCl, 1 N HCl respectively NaOH.
Only in 1 N NaOH Halopemide is instable due to the hydrolysis of the amide bond in the reaction conditions used.

References

1. U. S. Patent app. n° 697, 813, W. Soudijn, I. van Wijngaarden, P. A. J. Janssen.

2. B. Belleau and G. Malek, J. Amer. Chem.Soc. 90, 1651 (1968).

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