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Synthesis and in-vitro/in-vivo evaluation of orally administered entacapone prodrugs

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Abstract

Entacapone is a new inhibitor of catechol-*O*-methyltransferase (COMT) that is used as an adjunct to L-dopa therapy in the treatment of Parkinson's disease. The bioavailability of orally administered entacapone is, however, relatively low (29–46 %). In this study we have prepared more lipophilic acyl and acyloxyacyl esters, an acyloxy alkyl ether and an alkyloxycarbonyl ester of entacapone, and we have evaluated them as potential prodrugs to enhance the oral bioavailability of entacapone. All the derivatives fulfilled prodrug criteria and released entacapone in human serum in-vitro. The oral bioavailability of monopivaloyl (**1a**) and dipivaloyl (**1b**) esters of entacapone were investigated further in rats. The lipophilicity of **1b** was high (log P_{app} 4.0 at pH 7.4) but its oral bioavailability was low (F = 0.6 %), most probably due to its low aqueous solubility. The monopivaloyl ester of entacapone (**1a**) had a higher lipophilicity (log P_{app} 0.80) than entacapone (log P_{app} 0.18) at pH 7.4 while maintaining an aqueous solubility equal to entacapone. However, oral bioavailability was not increased when compared with the parent drug entacapone (F = 7.0 % and 10.4 %, respectively).

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Introduction

Entacapone (Bäckström et al 1995) [(*E*)-2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl)propenamide] (**1**) is a new 3,4-dihydroxy-5-nitrobenzylidine derivative and a potent inhibitor of catechol-*O*-methyltransferase (COMT) (Bäckström et al 1989; Männistö et al 1992). L-Dopa (3,4-dihydroxyphenyl-L-alanine) is mainly metabolized by COMT and aromatic amino acid decarboxylase (AADC) (Männistö & Kaakkola 1990). L-Dopa is a precursor to dopamine, of which there is a shortage in the brains of patients suffering from Parkinson's disease.

Traditional Parkinson's disease treatment consists of L-dopa administration combined with an AADC inhibitor. During treatment, COMT remains the main enzyme to metabolize L-dopa although some new pathways may be activated. Even after this combination therapy, no more than 5–10 % of the orally administered L-dopa enters the brain (Männistö & Kaakkola 1990). The administration of entacapone, together with L-dopa and an AADC inhibitor, leads to increased L-dopa bioavailability and prolonged elimination of L-dopa (Kaakkola et al 1994).

Entacapone is used clinically as an adjunct to L-dopa therapy in Parkinson's disease (Männistö & Kaakkola 1999); however, the bioavailability of entacapone is low (29–46 %) after oral administration and characterized by large inter-

individual variation (Keränen et al 1994). The low oral bioavailability of entacapone may be due to several reasons. Firstly, entacapone (weak acid; pK_a 4.5) is unionized at low pH (i.e. in the stomach) with poor aqueous solubility and slow dissolution, which may limit its absorption (Savolainen et al 2000a). Secondly, at higher pH (i.e. in the small intestine) entacapone is mostly ionized and its solubility and dissolution properties are improved. Unfortunately, ionized drug molecules having low lipophilicity are generally poorly absorbed from the small intestine (Lee et al 1997). Thirdly, high first-pass metabolism of phenols and catechols may decrease the bioavailability of entacapone (Wikberg et al 1993; Friis & Bundgaard 1996).

The aim of the study was to investigate whether the oral bioavailability of entacapone could be improved by lipophilic prodrugs. Therefore, we synthesized acyl and acyloxyacyl esters, an acyloxy alkyl ether and an alkyoxycarbonyl ester of entacapone and evaluated the derivatives as potential prodrugs of entacapone.

Materials and Methods

Materials

Entacapone ((*E*)-2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl)propenamide, OR-611), Z-entacapone and tolcapone (4′-methyl-3,4-dihydroxy-5-nitrobenzophenone, Ro 40-7592) were obtained from Orion Pharma (Espoo, Finland). All other materials and solvents were commercially available and used as received. All melting points are uncorrected. Structures of the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and electrospray ionization mass spectrometry (ESI-MS). ^{1}H and ^{13}C NMR spectra were obtained by a Bruker AM 400 WB spectrometer (operating at 400.14 and 100.6 MHz, respectively) using $CDCl_3$ as a solvent and tetramethylsilane as internal reference. The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, qv = quintet, sx = sextet, br = broad. Electrospray ionization mass spectra were acquired using a LCQ ion trap mass spectrometer equipped with an electrospray ionization source (Finnigan MAT, San Jose, CA). The samples were diluted with methanol to 20 μg mL^{-1} and injected directly to the eluent flow via a 5-μL loop injector (the total amount of the sample was approximately 100 ng). Full scan mass spectra were recorded. The synthesis reactions were monitored by TLC (Kieselgel 60 F 254, DC-Alufolien, Merck). The synthesis products were purified without attempts to maximize yields.

Synthesis methods

Method A

Entacapone (1.0 g, 3.3 mmol) was dissolved in dry pyridine (7 mL) and cooled to 0°C when valeroyl chloride (1.2 g, 8.3 mmol) was added to the mixture. The mixture was stirred at room temperature for 15 h, the pyridine hydrochloride was filtered off and the filtrate was evaporated. The residue was purified by column chromatography using ethyl acetate/petroleum ether (10:1) as an eluent to give (*E*)-2-cyano-*N*,*N*-diethyl-3-[3,4-divaleroyloxy-5-nitrophenyl]propenamide (**1f**) (0.17 g, 11% of theoretical), as a clear yellowish oil. ^{1}H NMR δ: 0.91 (3H, $J = 7.3$ Hz, t, $CH_2CH_2CH_3$), 0.97 (3H, $J = 7.3$ Hz, t, $CH_2CH_2CH_3$), 1.24 (6H, br, NCH_2CH_3), 1.35 (2H, $J = 7.4$ Hz, m, $CH_2CH_2CH_3$), 1.46 (2H, $J = 7.4$ Hz, m, $CH_2CH_2CH_3$), 1.61 (2H, $J = 7.5$ Hz, m, $CH_2CH_2CH_2$), 1.77 (2H, $J = 7.5$ Hz, m, $CH_2CH_2CH_2$), 2.34 (2H, $J = 7.4$ Hz, t, $COCH_2$), 2.65 (2H, $J = 7.4$ Hz, t, $COCH_2$), 3.48 (4H, br, NCH_2), 7.58 (1H, s, $CH{=}C$), 8.06 (1H, $J = 2.2$ Hz, d, ArH[2]), 8.43 (1H, $J = 2.2$ Hz, d, ArH[6]). ^{13}C NMR δ: 12.9, 13.0, 13.6, 17.8, 18.2, 35.5, 40.8, 43.4, 110.9, 114.7, 124.1, 128.5, 130.3, 139.0, 143.0, 144.8, 146.2, 161.8, 169.4, 170.1. ESI-MS: 474.0 (M + 1).

Method B

Entacapone (1.5 g, 4.9 mmol), dimethylaminopyridine (DMAP) (15 mg) and valeroyloxy acetic acid (3.2 g, 19.7 mmol) were dissolved in dry tetrahydrofuran (30 mL). Dicyclohexylcarbodiimide (DCC) (2.1 g, 10.1 mmol) was added and the solution was stirred at room temperature overnight. The mixture was filtered and the filtrate was evaporated. The residue was purified by column chromatography using ethyl acetate/hexane 1:1 as an eluent to give (*E*)-2-cyano-*N*,*N*-diethyl-3-[3,4-divaleryloxyacetyloxy-5-nitrophenyl]propenamide (**2c**) (1.5 g, 52% of theoretical) as a clear yellow oil. ^{1}H NMR δ: 0.86 (6H, $J = 7.6$ Hz, t, CH_2CH_3), 1.25 (6H, br, NCH_2CH_3), 1.36 (4H, $J = 7.7$ Hz, sx, CH_2CH_3), 1.63 (4H, $J = 7.7$ Hz, qv, $CH_2CH_2CH_2$), 2.39 (4H, $J = 7.6$ Hz, t, CH_2CO), 3.44 (4H, br, NCH_2), 4.90 (2H, s, O-CH_2-CO), 4.93 (2H, s, O-CH_2-CO), 7.55 (1H, s, $CH{=}C$), 8.01 (1H, $J = 2.2$ Hz, d, ArH[2]), 8.47 (1H, $J = 2.2$ Hz, s, ArH[6]). ^{13}C NMR δ: 12.6, 13.8, 14.3, 22.1, 26.9, 33.5, 41.1, 43.7, 60.3, 60.8, 108.8, 115.5, 124.6, 128.9, 130.2, 140.6, 142.8, 147.3, 149.9, 162.6, 164.9, 165.6, 173.3, 173.4. ESI-MS: 590.0 (M + 1).

Method C

Entacapone (1.0 g, 3.3 mmol) was added to pivaloyl anhydride (5 mL containing a drop of conc. H_2SO_4) and the mixture was stirred at 50°C for 2 h. The mixture was poured into water and extracted twice with diethyl ether. The organic phase was washed with 5% $NaHCO_3$, dried with Na_2SO_4 and evaporated. The residue was purified by recrystallization from ethyl acetate/petroleum ether to give (*E*)-2-cyano-*N*,*N*-diethyl-3-[3-pivaloyloxy-4-hydroxy-5-nitrophenyl]propenamide (**1a**) (0.62 g, 48% of theoretical) as a yellow crystalline powder; mp 125.3°C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (6H, br, CH_2CH_3), 1.41 (9H, s, CCH_3), 3.50 (4H, br, NCH_2), 7.59 (1H, s, CH=C), 8.03 (1H, J = 2.2 Hz, d, ArH[2]), 8.46 (1H, J = 2.2 Hz, d, ArH[6]), 10.9 (1H, br, ArOH). ^{13}C NMR δ: 12.7, 13.65, 27.1, 39.35, 41.0, 43.5, 108.1, 115.4, 123.8, 124.6, 129.9, 134.3, 141.9, 147.3, 150.3, 162.4, 175.9. ESI-MS: 390.0 (M + 1).

In the following syntheses, 3.3 mmol (1.0 g) entacapone was used as the starting material.

(E)-2-Cyano-N,N-diethyl-3-[3,4-dipivaloyloxy-5-nitrophenyl]propenamide (1b)

Method A

Yield 0.69 g (45% of theoretical); mp 106.5°C. Exceptions in the method: 4 equiv. pivaloyl chloride and 10 mg dimethylaminopyridine were used. ^{1}H NMR δ: 1.26 (6H, br, NCH_2CH_3), 1.41 (18H, s, CCH_3), 3.49 (4H, br, NCH_2), 7.59 (1H, s, CH=C), 7.99 (1H, J = 2.2 Hz, d, ArH[2]), 8.33 (1H, J = 2.2 Hz, d, ArH[6]). ^{13}C NMR δ: 12.3, 13.7, 26.4, 27.0, 39.2, 39.3, 40.0, 43.3, 112.8, 115.8, 123.1, 128.7, 130.0, 138.9, 141.7, 143.3, 145.1, 160.3, 174.3, 175.2. ESI-MS: 474.0 (M + 1).

(E)-2-Cyano-N,N-diethyl-3-[3,4-dibutyryloxy-5-nitrophenyl]propenamide (1e)

Method A

Yield 1.36 g (93% of theoretical); oil. Exceptions in the method: 4 equiv. butyroyl chloride and 4 equiv. triethylamine in dry tetrahydrofuran were used. ^{1}H NMR δ: 1.04 (6H, J = 7.2 Hz, t, $CH_2CH_2CH_3$), 1.24 (6H, br, NCH_2CH_3), 1.76 (2H, J = 7.2 Hz, m, $CH_2CH_2CH_3$), 1.80 (2H, J = 7.2 Hz, m, $CH_2CH_2CH_3$), 2.55 (2H, J = 7.3 Hz, t, $COCH_2$), 2.60 (2H, J = 7.3 Hz, t, $COCH_2$), 3.48 (4H, br, NCH_2), 7.60 (1H, s, CH=C), 8.07 (1H, J = 2.1 Hz, d, ArH[2]), 8.32 (1H, J = 2.1 Hz, d, ArH[6]). ^{13}C NMR δ: 12.9, 13.0, 17.8, 18.2, 35.5, 40.8, 43.4, 110.9, 114.7, 124.1, 128.5, 130.3, 139.0, 143.0, 144.8, 146.2, 161.8, 169.4, 170.1. ESI-MS: 446.0 (M + 1).

(E)-2-Cyano-N,N-diethyl-3-[3,4-dibenzoyloxy-5-nitrophenyl]propenamide (1d)

Method A

Yield 0.14 g (10% of theoretical); mp 143.0°C. Exceptions in the method: 2.5 equiv. benzoyl chloride was used (dimethylaminopyridine was not used). ^{1}H NMR δ: 1.24 (6H, br, NCH_2CH_3), 3.52 (4H, br, NCH_2), 7.40 (4H, m, ArH), 7.56 (2H, m, ArH), 7.66 (1H, s, CH=C), 8.04 (4H, m, ArH), 8.28 (1H, J = 2.2 Hz, d, ArH[2]), 8.45 (1H, J = 2.2 Hz, d, ArH[6]). ^{13}C NMR δ: 12.77, 14.01, 41.1, 43.7, 111.4, 115.1, 124.5, 127.5, 127.7, 128.9, 128.9, 130.6, 130.6, 130.9, 134.7, 134.7, 139.9, 143.6, 145.6, 146.6, 162.2, 163.1, 163.8. ESI-MS: 514.1 (M + 1).

(E)-2-Cyano-N,N-diethyl-3-[3,4-diacetoxyacetyloxy-5-nitrophenyl]propenamide (2a)

Method A

Yield 0.23 g (28% of theoretical); oil. Exceptions in the method: 1.6 mmol entacapone, 4 equiv. acetoxyacetyl chloride and 4 equiv. triethylamine in tetrahydrofuran were used (dimethylaminopyridine was not used). ^{1}H NMR δ: 1.24 (6H, br, NCH_2CH_3), 2.17 (6H, s, CH_3CO), 3.54 (4H, br, NCH_2), 4.96 (4H, s, OCH_2CO), 7.60 (1H, s, CH=C), 8.06 (1H, J = 2.2 Hz, d, ArH[2]), 8.43 (1H, J = 2.2 Hz, d, ArH[6]). ^{13}C NMR δ: 12.4, 13.8, 20.2, 42.1, 43.0, 60.2, 60.3, 111.7, 114.7, 124.4, 128.6, 131.3, 138.8, 142.8, 144.3, 145.8, 161.7, 164.5, 165.2, 170.3. ESI-MS: 506.1 (M + 1).

(E)-2-Cyano-N,N-diethyl-3-[(3,4-diacetyloxy-5-nitrophenyl)]propenamide (1c)

Method C

Yield 0.95 g (74% of theoretical); mp 135.0°C. Exceptions in the method: 4 equiv. acetic acid anhydride dissolved in ethyl acetate was used. ^{1}H NMR δ: 1.26 (6H, d, NCH_2CH_3), 2.36 (3H, s, CH_3CO), 2.39 (3H, s, CH_3CO), 3.49 (4H, br, NCH_2), 7.61 (1H, s, CH=C), 8.1(1H, J = 2.2 Hz, d, ArH[2]), 8.35 (1H, J = 2.2 Hz, d, ArH[6]). ^{13}C NMR δ: 12.4, 13.6, 20.2, 20.3, 40.7, 43.3, 111.0, 114.7, 124.1, 128.4, 130.4, 138.8, 142.8, 144.7, 146.0, 161.8, 166.8, 167.5. ESI-MS: 389.9 (M).

(E)-2-Cyano-N,N-diethyl-3-[3,4-dipivaloyloxyacetyloxy-5-nitrophenyl]-propenamide (2b)

Method B

Yield 0.70 g (85% of theoretical); oil. Exceptions in the method: 1.4 mmol entacapone and 3.1 mmol piva-

loyloxy acetic acid and ethyl acetate as a solvent were used. ^{1}H NMR δ: 1.25 (6H, br, NCH_2CH_3), 1.38 (9H, s, CCH_3), 1.39 (9H, s, CCH_3), 2.17 (6H, s, CH_3CO), 3.50 (4H, br, NCH_2), 4.72 (2H, s, O-CH_2-CO), 4.82 (2H, s, OCH_2CO), 7.62 (1H, s, $CH=C$), 8.04 (1H, $J = 2.2$ Hz, d, Ar*H*[2]), 8.35 (1H, $J = 2.2$ Hz, d, Ar*H*[6]). ^{13}C NMR δ: 12.5, 13.4, 27.3, 39.6, 41.1, 43.0, 60.4, 61.0, 111.1, 115.1, 124.1, 128.7, 130.4, 139.8, 145.5, 146.7, 153.2, 132.2, 167.4, 167.6, 175.6. ESI-MS: 590.0 (M + 1).

(E)-2-Cyano-N,N-diethyl-3-[3,4-dipivaloyloxy(methoxy)-5-nitrophenyl]propenamide (3a)

Chloromethyl pivalate (0.75 g, 5 mmol) was dissolved in dry acetone (20 mL) and sodium iodide (0.75 g, 5 mmol) was added. The mixture was placed in the dark and stirred at room temperature for 3 h. Entacapone (0.75 g, 2.5 mmol) and potassium carbonate (1.4 g, 10 mmol) were added and stirring was continued overnight. The mixture was filtered and the filtrate was evaporated. The residue was purified by column chromatography using ethyl acetate as an eluent to give **3a** (1.5 g, 56 %) as a clear yellow oil. ^{1}H NMR δ: 1.15 (9H, s, CCH_3), 1.19 (9H, s, CCH_3), 1.27 (6H, br, NCH_2CH_3), 3.48 (4H, br, NCH_2), 5.77 (2H, s, O-CH_2-O), 5.83 (2H, s, O-CH_2-O), 7.55 (1H, s, $CH=C$), 7.82 (1H, $J = 2.2$ Hz, d, Ar*H*[2]), 8.1 (1H, $J = 2.2$ Hz, d, Ar*H*[6]). ^{13}C NMR δ: 12.6, 13.8, 26.8, 39.0, 39.2, 40.9, 43.3, 85.7, 88.8, 109.4, 115.3, 119.2, 120.2, 128.6, 143.6, 144.4, 147.3, 150.6, 162.2, 177.0, 177.7. ESI-MS: 556.1 ($M + 1 + Na^+$).

(E)-2-Cyano-N,N-diethyl-3-[3-t-butyloxycarbonyloxy-4-hydroxy-5-nitrophenyl]-propenamide (4a)

Entacapone (1.0 g, 3.3 mmol), sodium carbonate (0.7 g, 6.6 mmol), 15-crown-5 (catalytic amount) and di-tert-butyldicarbonate (0.72 g, 3.3 mmol) were dissolved in tetrahydrofuran (20 mL) and stirred for 12 h at 50°C. The mixture was filtered and the filtrate was evaporated to dryness in-vacuo. The product was recrystallized from petroleum ether/ethyl acetate to give **4a** (0.87g, 64 %) as a yellow powder. ^{1}H NMR δ: 1.25 (6H, br, NCH_2CH_3), 1.52 (9H, s, CCH_3), 3.50 (4H, br, NCH_2), 7.50 (1H, s, $CH=C$), 7.84 (1H, $J = 2.2$ Hz, d, Ar*H*[2]), 8.3 (1H, $J = 2.2$ Hz, d, Ar*H*[6]). ^{13}C NMR δ: 12.4, 13.3, 28.1, 42.0, 43.0, 84.2, 98.5, 113.3, 118.5, 124.5, 131.3, 139.8, 148.1, 151.8, 153.0, 164.2, 167.0. ESI-MS: 427.9 ($M + Na^+$).

Determination of aqueous solubilities

The aqueous solubilities of entacapone and its prodrugs were determined in phosphate buffer solution (0.16 M, pH 5.0 and 7.4) at room temperature. An excess amount of each compound was added to the buffered solution. The suspensions were shaken 30–60 min and filtered (Millipore 0.45 μm). Clear solutions were diluted, if necessary, and analysed by HPLC. All determinations were made in triplicate. The pH of the suspension was held constant throughout the study.

Determination of apparent partition coefficients

The apparent partition coefficient (P_{app}) of entacapone and **1a** were determined from the distribution of the compound between a mixture of 1-octanol and phosphate buffer (0.16 M), where the buffer solution and 1-octanol were saturated before the experiment by shaking overnight. A known concentration of derivative in phosphate buffer solution was shaken for 30 (**1a**) or 60 min (entacapone) with a suitable volume of the 1-octanol buffer solution. The phases were separated by centrifugation (3 min at 1500 rev min^{-1}) and the concentrations of the derivative were determined from the buffer phase by HPLC. Log P_{app} values of poorly water-soluble derivatives (**1b–e**, **2b** and **3a**) were determined from capacity factors (k′) by reversed-phase HPLC (Lambert 1993). A Purospher C18e column was used as the stationary phase and the mobile phase consisted of 25 mM phosphate/10 mM citrate buffer (pH 2.2) and methanol (28:72, v/v). The flow rate (isocratic) was 1.0 mL min^{-1} at 40°C. Column effluent was monitored at 254 nm by UV detection. The calibration curve was performed by using seven non-ionizable standard compounds. The standard compounds and their log P values were as follows: anisole 2.11 (Minick et al 1988), anthracene 4.54 (Hsieh & Dorsey 1995), budesonide 2.31 (Corwin et al 1990), hydrocortisone 1.53 (Singh & Roberts 1996), naphthalene 3.30 (Minick et al 1988), nitrobenzene 1.85 (Minick et al 1988) and progesterone 3.87 (Singh & Roberts 1996). The log P_{app} values of the standard compounds were plotted against the measured log k′ values and a linear relationship ($r^2 = 0.938$) was found between these two parameters. The log P_{app} values of entacapone prodrugs were determined from this linear relationship.

Hydrolysis in aqueous solution

Chemical hydrolysis rates of the entacapone prodrugs were determined in phosphate buffer (0.16 M) solution

at pH 3.0 (**1a–b**, **3a**) and pH 7.4 (all prodrugs) at 37°C. Solutions were prepared by dissolving an appropriate amount of compound in preheated (37°C) buffer solution. Due to the low aqueous solubility of some compounds, an addition of 5–10% ethanol was needed to achieve a detectable concentration. Samples were withdrawn at designated time intervals and analysed for remaining prodrug by HPLC. Pseudo-first order half-lives ($t_{\frac{1}{2}}$) for the hydrolysis of the derivatives were calculated from the linear slopes of logarithmic plots of remaining entacapone prodrug over time.

Hydrolysis in human serum

The rate of enzymatic hydrolysis for the prodrugs was determined in human serum diluted to 80% with 0.16 M phosphate buffer (pH 7.4) at 37°C. An appropriate amount of prodrug was dissolved in preheated buffer solution and preheated serum was added. Solutions were kept in a water bath at 37°C. At designated time intervals, samples of 0.5 mL were withdrawn and 1.0 mL ethanol was added to each sample to terminate enzymatic activity. After immediate mixing and centrifugation for 10 min at 14000 rev min^{-1}, the clear supernatant was analysed by HPLC for remaining prodrug and released entacapone. Pseudo-first order half-lives ($t_{\frac{1}{2}}$) for the hydrolysis of the derivative were calculated from the linear slopes of logarithmic plots of remaining entacapone prodrug over time.

Analytical procedure for the in-vitro samples

The HPLC system used for the determination of in-vitro samples consisted of a Beckman System Gold Programmable Solvent Module 126, a Beckman System Gold Detector Module 166 with variable wavelength UV detector (set at 254–304 nm, depending on compound) and a Beckman System Gold Autosampler 507e. Separations were performed on a Purospher RP-18e reverse-phase column (12.5 cm × 4.0 mm i.d., 5 μm). The chromatographic conditions were as follows: loop injection volume, 50 μL; column temperature, 40°C; flow rate, gradient/isocratic at 1.0 mL min^{-1}. The mobile phase consisted of various percentages of a methanol/water mixture (90:10) and citrate/phosphate buffer (pH 2.2).

Preparation of dosage forms

Suspensions of entacapone and selected prodrugs (**1a–b**) were prepared by weighing an appropriate amount of compound, after which potassium biphthalate buffer (pH 3.0, USP 23) was added. Suspensions were sonicated to obtain homogenous solutions. The entacapone intravenous solution (pH 7.4) was prepared by dissolving entacapone in a phosphate buffer solution (pH 7.4; 50 mM). The solution was filtered through a sterile membrane (pore size 0.22 μm) before making it isotonic by the addition of an amount of NaCl. Entacapone content of all preparations was 3.73×10^{-6} mol mL^{-1}.

In-vivo absorption studies

Male Han/Wistar rats (7–9-weeks-old, 140–270 g; National Laboratory Animal Centre, University of Kuopio) were housed in stainless steel cages and kept on a 12-h light–dark cycle (lights on at 0700 h) at an ambient temperature of 22 ± 1°C. The relative air humidity was 50 ± 10%. Pelleted food (Lactamin R36, Lactamin AB, Södertälje, Sweden) was removed 24 h before the experiment but water was freely available.

Entacapone and prodrugs (doses equal to 5.7 mg kg^{-1} entacapone) were administered orally by gavage in a volume of 0.5 mL/100 g animal weight. Entacapone was also administered intravenously to a tail vein (0.5 mL/100 g). Vasodilatation of tail veins was induced by warm water just before intravenous injection.

Rats were decapitated 2.5, 5, 10, 20, 30, 60 and 120 min (i.v.) or alternatively 10, 20, 30, 60 and 120 min (p.o.) after drug administration. Blood samples were collected in glass tubes containing EDTA to prevent coagulation. Plasma was separated by centrifugation at +4°C at 1500 *g* for 10 min. Plasma samples were transferred into plastic tubes and stored at −80°C until analysed. All procedures with animals were reviewed and approved by the Animal Ethics Committee at the University of Kuopio.

Analytical procedure for the in-vivo samples

The HPLC used for the determination of in-vivo samples was similar to the one used for in-vitro samples, except that the mobile phase contained a water/acetonitrile mixture instead of water/methanol mixture. A linear gradient elution was used and the mobile phase consisted of various percentages of an acetonitrile/water mixture (0–7 min 40% acetonitrile; 7–10 min 48% acetonitrile; 10–20 min 87% acetonitrile) and citrate/phosphate buffer (pH 2.2).

Before extraction, 100 μL 2 M hydrochloric acid was added to 1.0 mL plasma and 50 μL of a tolcapone solution was added as an internal standard. Entacapone, Z-entacapone, prodrug and internal standard were extracted from plasma using Bond Elut C18 (3 CC/500 mg) solid phase extraction cartridges (Analytichem International, Harbor City, CA) and 2.5 mL acetonitrile as an eluent. The eluent was evaporated and the residues were dissolved in 300 μL of a water/methanol mixture

(50:50) and injected onto the HPLC. The results were calculated from peak-area ratios.

A standard curve was prepared for entacapone by mixing blank plasma with a known amount of entacapone (62.7–1960 ng mL^{-1}) and internal standard (tolcapone). The standard curve showed excellent linearity ($r^2 = 0.999$) which made one-point calibration feasible. Separate spiked plasma standard samples containing Z-entacapone and two concentrations of entacapone and spiked plasma samples containing two concentrations of prodrug were prepared daily. All the spiked plasma samples contained tolcapone as an internal standard.

Intraday precision (coefficient of variation) of the method was assessed by extracting and analysing plasma samples, containing entacapone and prodrugs (**1a** and **1b**), four to six times in one day. The intraday precision was 1.4, 3.9 and 6.3% for entacapone, **1a** and **1b**, respectively.

In-vivo data analysis

The maximum plasma concentrations (C_{max}) and the amount of time required to reach the maximum (t_{max}) were obtained directly from the plasma concentration vs time data. The entacapone concentration in plasma (C) after an intravenous administration was best described by the biexponential equation, $C = Ae^{-\lambda 1t} + Ae^{-\lambda 2t}$, where t is time. Results were obtained with the Kaleida Graph (version 3.0.1) program (Macintosh). Areas under the concentration vs time curves from 0 to infinity ($AUC_{0-\infty}$) following intravenous injection were estimated by using the equation $AUC_{0-\infty} = A_1/\lambda_1 + A_2/\lambda_2$ (Gibaldi & Perrier 1982). For oral dosage forms, $AUC_{2h-\infty}$ was the sum of AUC_{0-2h} and $AUC_{2h-\infty}$, where AUC_{0-2h} was calculated by using the linear trapezoidal method and $AUC_{2h-\infty}$ was estimated by dividing entacapone concentration at 2-h post-dosing by λ_2 (Gibaldi & Perrier 1982). The calculation of the bioavailability of prodrugs was based on the sum of entacapone and prodrug concentration in plasma.

A one-factor analysis of variance (for factorial measurements) was used to test the statistical significance of differences between formulations at each time point. Significance in the differences of the means was tested using Fisher's protected least significant difference (PLSD) method at the 95% confidence level.

Results and Discussion

Chemistry

The synthetic routes are summarized in Figure 1. Acyl esters **1a–f** were synthesized from their corresponding acid chlorides and carboxylic acid anhydrides. The structure of **1a** was confirmed by X-ray crystallography, which showed that the pivaloyl moiety was at the C-3 hydroxy position (Leppänen et al 2001). Acyl migration to the neighbouring hydroxyl group was not observed (Ihara et al 1990).

Acyloxyacyl esters **2a–b** were synthesized from acid chlorides in the presence of pyridine or via the route that employed dicyclohexylcarbodiimide (DCC) in THF (**2c**). The synthesis of acyloxymethyl ether **3a** was found to be sensitive to moisture and the treatment of chloromethyl pivaloate with NaI was essential, otherwise an unwanted acylated product (**1a**) was obtained (Bodor et al 1983). Attempts to synthesize a monosubstituted acyloxymethyl ether primarily resulted in an acylated product. t-Butyloxycarbonyl ester **4a** was synthesized from $(Boc)_2O$ using Na_2CO_3 and 15-crown-5 as a catalyst. Similarly to **1a** entacapone was substituted merely to the C-3 position.

Promoieties were primarily allowed to react only to the other hydroxyl group of entacapone (**1a**, **4a**) to maintain adequate aqueous solubility and to increase lipophilicity.

Unfortunately these derivatives have low chemical stability at pH 7.4, which probably follows from the intramolecular hydrolysis initiated by the unsubstituted hydroxyl group. Therefore further syntheses of similar derivatives were not carried out. Due to the low aqueous solubilities of more stable disubstituted derivatives (e.g. **1b–f**), amino acid derivatives were synthesized to maintain the stability and to increase aqueous solubility. L-Isoleucine and glycine have been used successfully to improve the bioavailability of penciclovir (Kim et al 1999) and their Boc-protected derivatives of entacapone were synthesized. However, after the cleavage of the protective group these derivatives were very labile (Jensen et al 1991) and were not evaluated for prodrug purposes.

In some reactions (**1b**, **1d**, **2b** and **3a**) the parent compound was partially converted to its *Z*-form. The mechanism of the conversion and the chemical environment behind this process were not investigated in this study.

Aqueous solubilities and apparent partition coefficients

The aqueous solubilities and apparent partition coefficients of entacapone and its prodrugs **1a** and **4a** are listed in Table 1. The aqueous solubilities of **1b–f**, **2a–c** and **3a** were not determined due to their low aqueous solubility. Their apparent partition coefficients were

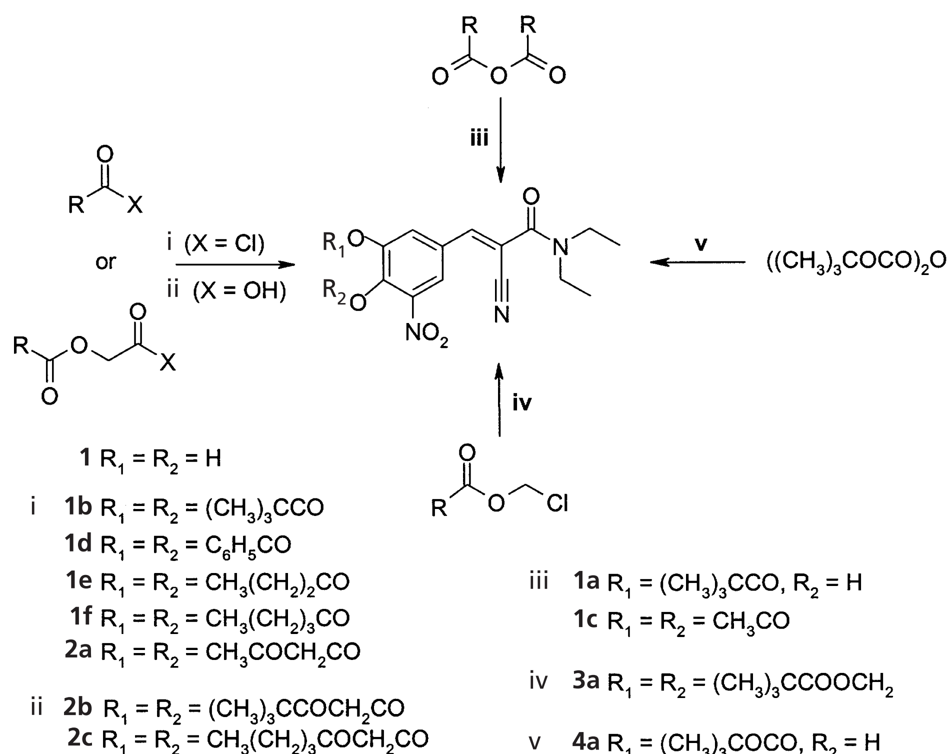

Figure 1 Synthesis routes of entacapone prodrugs. i: acid chloride, pyridine, (DMAP); ii: carboxylic acid, DCC, THF, DMAP; iii: acid anhydride, reflux; iv: 1. acyloxymethyl chloride, NaI, acetone; 2. K_2CO_3, **1**; iv: $(Boc)_2O$; Na_2CO_3, 15-Crown-5, THF.

Table 1 Apparent partition coefficients (log P_{app}) and aqueous solubilities of entacapone (**1**) and its prodrugs **1a** and **4a**.

Compound	log P_{app}		
	pH 5.0[a]	pH 7.4[a]	With column[b]
1	1.58±0.01	0.18±0.03	0.65
1a	2.24±0.01	0.80±0.06	–
1b	–	–	4.00
1c	–	–	1.23
1d	–	–	3.44
1e	–	–	3.15
2b	–	–	3.99
3a	–	–	3.86
	Aqueous solubility (μg mL^{-1})[a,c]		
	pH 5.0	pH 7.4	
1	77.3±2.3	1751.8±119.1	
1a	5.2±0.6	1623.0±76.0	
4a	–	6993.0±792.0	

[a]Values were mean±s.d., n = 3. [b]Apparent partition coefficients were determined by using the capacity factor of reversed phase HPLC. [c]The aqueous solubilities of the other prodrugs were not determined due to low chemical stability or low aqueous solubility (below detection limit of the HPLC method).

determined by using the capacity factor of reversed-phase HPLC. Log P_{app} of **1f**, **2a**, **2c** and **4a** was not determined due to inadequate chemical stability (Lambert 1993).

The aqueous solubility of entacapone was very low at acidic pH (e.g. 17 μg mL^{-1} at pH 1.2) but improved with increasing pH (e.g. 1750 μg mL^{-1} at pH 7.4) (Savolainen et al 2000b). The large difference in the aqueous solubility of entacapone was due to its low pK_a value (4.5) (Wikberg 1993).

The monosubstituted entacapone prodrug **1a** had a higher log P_{app} value than entacapone, while adequate aqueous solubility was maintained at pH 7.4 (Table 1). However, the aqueous solubility of **1a** decreased more rapidly with decreasing pH when compared with entacapone. Compound **4a** improved aqueous solubility threefold compared with entacapone.

Chemical and enzymatical hydrolysis

The degradation kinetics of the prodrugs followed first-order kinetics, and their half-lives ($t\frac{1}{2}$) are presented in Table 2.

The chemical stability of the prodrugs was higher at low pH than at neutral pH. In contrast, carbamate

Table 2 Half-lives of entacapone prodrugs in phosphate buffer (0.16 M) and 80% human serum (pH 7.4) at 37°C.

Compound	$t_{\frac{1}{2}}$ (h) Phosphate buffer			$t_{\frac{1}{2}}$ (h) Human serum
	pH 3.0	pH 5.0	pH 7.4	pH 7.4
1a	11.1	2.0	2.1	0.72
1b	87.5	71.6	7.6	[c]
1c	[a]	7.2	0.4	[c]
1d	[a]	4.1	4.8	[c]
1e	[a]	[a]	1.6	[c]
1f	[a]	[a]	[b]	[c]
2a	[a]	0.07	[b]	[c]
2b	[a]	58.1	18.7	[c]
2c	[a]	[a]	[b]	[c]
3a	197.5	41.5	43.4	0.9[d]
4a	[a]	[a]	0.7	1.0

[a]Not determined. [b]Degraded instantly to entacapone. [c]Released entacapone, but the kinetics could not be determined due to low aqueous solubility of the compound. [d]Release of entacapone was incomplete.

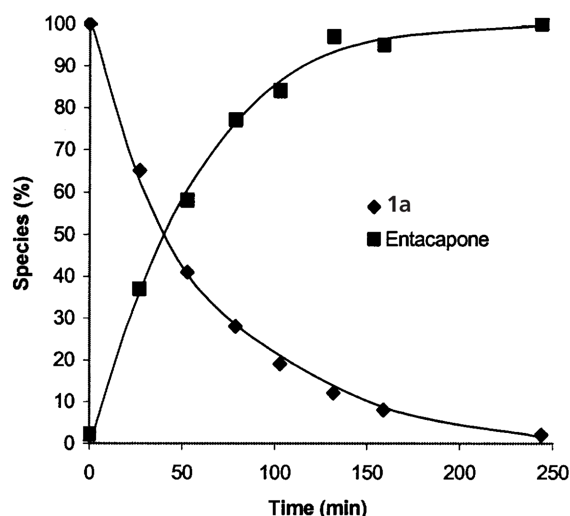

Figure 2 Bioconversion of **1a** to entacapone in 80% human serum (pH 7.4) at 37°C.

prodrugs of entacapone had the opposite behaviour and hydrolysed faster at low pH than at neutral pH (Savolainen et al 2000b). The increased stability of the prodrugs in an acidic environment was a desired property because they were protected against chemical hydrolysis at the acidic pH (i.e. stomach).

The branched prodrugs **1a**, **1b**, **2b** and **3a** were more stable against chemical hydrolysis than prodrugs **1e** and **1f** (Table 2), which was in good agreement with earlier prodrug studies (Chien et al 1991; Geraldine et al 1992). Diacyl ester prodrugs are chemically more stable than monoacyl esters, due to the lacking intramolecular hydrolysis. The carbonate structure of **4a** was less stable than ester structure **1a** at pH 7.4, which may limit its usefulness because entacapone might be released before absorption in the small intestine.

Chemical stability of the acyloxy acyl esters **2a–c** was very low. The most branched (**2b**) was the most stable. The higher stability of **2b** suggested that the chemical hydrolysis rate of acyloxy acyl esters was mostly dependent on the second ester group of the promoiety.

Prodrug **3a** had the highest chemical stability due to the location of the ester group far from the electron withdrawing nitrocatechol structure via a methyl spacer. This stabilization was reflected in the half-lives of the acyl esters **1b** and **3a**, which were 7.6 and 43.4 h at pH 7.4, respectively.

All entacapone prodrugs released entacapone in 80% human plasma and thus fulfilled the prodrug criteria.

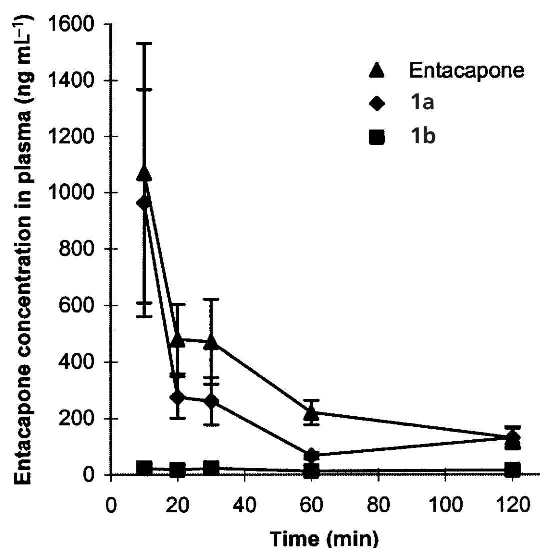

Figure 3 The concentration of entacapone in plasma after oral administration of entacapone, **1a** and **1b** as suspensions (pH 3.0) to rats (mean value, $n \geqslant 4$). The administered dose was always equivalent to 5.7 mg kg^{-1} entacapone.

However, due to their low aqueous solubility, accurate values of enzymatic hydrolysis rates were successfully determined for **1a**, **3a** and **4a** only. Prodrugs **1a** (Figure 2) and **4a** hydrolysed quantitatively to the parent entacapone in serum, but in the case of **3a** only 20% of the parent drug was released after complete degradation of **3a**. The reason for the incomplete bioconversion of **3a**

Table 3 Mean pharmacokinetic parameters of entacapone in plasma after oral administration of entacapone and its prodrugs and after intravenous administration of entacapone to rats ($n \geqslant 4$). Administered dose was always equivalent to 5.7 mg kg^{-1} entacapone.

Formulation	C_{max} (ng mL^{-1})	t_{max} (min)	AUC (0–2 h) (ng h mL^{-1})	AUC (0–∞) (ng h mL^{-1})	F, (0–2 h) (%)	F, (0-∞) (%)
1 suspension[a]	⩾ 1062.0	⩽ 10	644.3	708.7	10.5	10.4
1a suspension[a]	⩾ 963.3	⩽ 10	409.0	476.7	6.7	7.0
1b suspension[a]	⩾ 23.0	⩽ 10	32.1	40.4	0.5	0.6
1 solution (i.v.)[b]	33496.2	2.5	6126.3	6806.6	100.0	100.0

[a]pH 3.0. [b]pH 7.4.

during a 4-h enzymatic hydrolysis reaction might have been due to a more stable intermediate prolonging the release of entacapone. The good chemical stability of **3a** made it a possible prodrug of entacapone, but its low aqueous solubility, together with the slow release of entacapone, decreased its usefulness.

Prodrugs **1a**, **3a** and **4a** were hydrolysed enzymatically at least twice as rapidly as entacapone prodrugs (Leppänen et al 2000; Savolainen et al 2000b). Rapid enzymatic hydrolysis to release entacapone immediately after the absorption enables effective COMT-inhibition in the small intestine where COMT-activity is high (Bäckström et al 1989).

Bioavailability in-vivo

The absolute bioavailability of **1a**, **1b** and entacapone was studied in rats, and the selection of the prodrugs was based on their in-vitro properties. Their chemical stability was adequate and they released entacapone in 80% human serum. The aqueous solubility of **1a** was comparable with the aqueous solubility of entacapone at pH 7.4, but its log P_{app} was higher when compared with entacapone. Prodrug **1b** had a markedly higher log P_{app} value when compared with entacapone.

The absolute bioavailability ($AUC_{0-\infty}$) of entacapone, **1a** and **1b** (administered orally as suspensions) were 10.4%, 7.0% and 0.6%, respectively (Figure 3 and Table 3). The poor bioavailability of **1b** was considered to be a result of its low aqueous solubility. In the case of **1b**, no prodrug was detected in the plasma after oral administration, but all of the detected drug was entacapone. This suggested that **1b** was partly degraded in the gastrointestinal-lumen and absorbed as entacapone.

The bioavailability of **1a** was slightly lower than that of entacapone. According to Figure 3 entacapone and **1a** had rapid parallel absorption followed by a rapid decrease. The site of absorption was thus mainly in the upper gastrointestinal-tract where pH was low and with decreasing pH the aqueous solubility of **1a** decreased more rapidly than that of entacapone and thus its bioavailability was reduced. Therefore an ideal promoiety of entacapone should have, in addition to better chemical stability, an ionizable or polar group to increase aqueous solubility at low pH.

Conclusions

The novel entacapone derivatives released the parent compound, and thus fulfilled the prodrug criteria. All disubstituted entacapone prodrugs were more lipophilic than the parent entacapone, but their low aqueous solubilities prevented their oral administration trials in rats.

The acyloxymethyl ether (**3a**) proved to be a useful synthetic promoiety for the nitrocatechol structure, as it was chemically more stable than the corresponding acyl esters and its enzymatical hydrolysis was fast. However, its poor aqueous solubility and incomplete bioconversion to entacapone might limit its usefulness.

Prodrug **1a** had a higher lipophilicity than entacapone and while retaining the adequate aqueous solubility equal to entacapone, its oral bioavailability in the rat was lower than that of entacapone. This study showed that lipophilic entacapone prodrugs were not able to improve oral bioavailability in the rat.

References

Bäckström, R., Honkanen, E., Pippuri, A., Kairisalo, P., Pystynen, J., Heinola, K., Nissinen, E., Linden, I.-B., Männistö, P. T., Kaakkola, S., Pohto, P. (1989) Synthesis of some novel potent and selective catechol-*O*-methyltransferase inhibitors. *J. Med. Chem.* **32**: 841–846

Bäckström, R. J., Heinola, K. E., Honkanen, E. J., Kaakkola, S. K., Kairisalo, P. J., Linden, I.-B. Y., Männistö, P. T., Nissinen, E. A. O., Pohto, P., Pippuri, A. K., Pystynen, J. J. (1995) Pharmacologically active catechol derivatives. US Patent 5446194

Bodor, N., Sloan, K. B., Kaminski, J. J., Shih, C., Pogany, S. (1983) A convenient synthesis of (acyloxy)alkyl alpha ethers of phenols. *J. Org. Chem.* **48**: 5280–5284

Chien, D.-S., Sasaki, H., Bundgaard, H., Buur, A., Lee, V. H. L. (1991) Role of enzymatic lability in the corneal and conjunctival penetration of timolol ester prodrugs in the pigmented rabbit. *Pharm. Res.* **8**: 728–733

Corwin, H., Sammes, P. G., Taylor, J. B., Drayton, C. J. (eds) (1990) *Comprehensive medicinal chemistry: the rational design, mechanistic study & therapeutic application of chemical compounds*. Oxford Pergamon Press, Oxford, pp 332

Friis, G. J., Bundgaard, H. (1996) Design and application of prodrugs. In: Krogsgaard-Larsen, P., Liljefors, T., Madsen, U. (eds) *A textbook of drug design and development*. Harwood Academic Publishers, Amsterdam, pp 374–375

Geraldine, C., Jordan, M., Quigley, J. M., Timoney, R. F. (1992) Synthesis, hydrolysis kinetics and lipophilicity of O-acyl esters of oxprenolol. *Int. J. Pharm.* **84**: 175–189

Gibaldi, M., Perrier, D. (eds) (1982) *Pharmacokinetics*. Marcel Dekker, New York

Hsieh, M.-M., Dorsey, J. G. (1995) Bioavailability estimation by reversed-phase liquid chromatography: high bonding density C-18 phases for modeling biopartitioning process. *Anal. Chem.* **67**: 48–57

Ihara, M., Nakajima, S., Hisaka, A., Tsuchiya, Y., Sakuma, Y., Suzuki, H., Kitani, K., Yano, M. (1990) Hydrolysis and acyl migration of a catechol monoester of L-dopa: L-3-(-3-hydroxy-4-pivaloyloxyphenyl)alanine. *J. Pharm. Sci.* **79**: 703–708

Jensen, E., Falch, E., Bundgaard, H. (1991) Water-soluble aminoalkylbenzoate esters of phenols as prodrugs. Synthesis, enzymatic hydrolysis and chemical stability of paracetamol esters. *Acta. Pharm. Nord.* **3**: 31–37

Kaakkola, S., Teräväinen, H., Ahtila, S., Rita, H., Gordin, A. (1994) Effect of entacapone, a COMT inhibitor, on clinical disability and levodopa metabolism in Parkinsonian patients. *Neurology* **44**: 77–80

Keränen, T., Gordin, A., Karlsson, M., Korpela, K., Pentikäinen, P. J., Rita, H., Schultz, E., Seppälä, L., Wikberg, T. (1994) Inhibition of soluble catechol-*O*-methyltransferase and single-dose pharmacokinetics after oral and intravenous administration of entacapone. *Eur. J. Clin. Pharmacol.* **46**: 151–157

Kim, D.-K., Lee, N., Kim, Y.-W., Chang, K., Im, G.-J., Choi, W.-S., Kim, K. H. (1999) Synthesis and evaluation of amino acid esters of 6-deoxypenciclovir as potential prodrugs of penciclovir. *Bioorg. Med. Chem.* **7**: 419–424

Lambert, W. J. (1993) Modeling oil-water partitioning and membrane permeation using reversed-phase chromatography. *J. Chromatogr. A.* **656**: 469–484

Lee, C.-P., de Vrueh, R. L. A., Smith, P. L. (1997) Selection of development candidates based on in vitro permeability measurements. *Adv. Drug Del. Rev.* **23**: 47–62

Leppänen, J., Huuskonen, J., Savolainen, J., Nevalainen, T., Taipale, H., Vepsäläinen, J., Gynther, J., Järvinen, T. (2000) Synthesis of a water-soluble prodrug of entacapone. *Bioorg. Med. Chem. Lett.* **10**: 1967–1969

Leppänen, J., Nevalainen, T., Järvinen, T., Wegelius, E., Gynther, J., Huuskonen, J. (2001) Structural studies of acyl ester prodrugs of entacapone. *J. Mol. Struct.* **562**: 129–135

Männistö, P. T., Kaakkola, S. (1990) Rationale for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. *Pharmacol. Toxicol.* **66**: 317–323

Männistö, P. T., Kaakkola, S. (1999) Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol. Rev.* **51**: 593–628

Männistö, P. T., Ulmanen, I., Lundström, K., Taskinen, J., Tenhunen, J., Tilgmann, C., Kaakkola, S. (1992) Characteristics of catechol-*O*-methyltransferase (COMT) and properties of selective COMT inhibitors. *Prog. Drug Res.* **39**: 291–350

Minick, D. J., Frentz, J. H., Patrick, M. A., Brent, D. A. A. (1988) Comprehensive method for determining hydrophobicity constants by reversed-phase liquid chromatography. *J. Med. Chem.* **31**: 1923–1933

Savolainen, J., Forsberg, M., Taipale, H., Männistö, P. T., Jarho, P., Järvinen, K., Gynther, J., Järvinen, T. (2000a) Effects of aqueous solubility and dissolution characteristics on oral bioavailability of entacapone. *Drug Dev. Res.* **49**: 238–244

Savolainen, J., Leppänen, J., Forsberg, M., Taipale, H., Nevalainen, T., Huuskonen, J., Gynther, J., Männistö, P. T., Järvinen, T. (2000b) Synthesis and in vitro/in vivo evaluation of novel oral *N*-alkyl- and *N*,*N*-dialkyl-carbamate esters of entacapone. *Life Sci.* **67**: 205–216

Singh, P., Roberts, M. S. (1996) Local deep tissue penetration of compounds after transdermal application: Structure-tissue penetration relationships. *J. Pharmacol. Exp. Ther.* **279**: 908–917

Wikberg, T. (1993) Biotransformation and Bioanalytics of Nitecapone and Entacapone. Academic Dissertation, Yliopistopaino, Helsinki.

Wikberg, T., Vuorela, A., Ottoila, P., Taskinen, J. (1993) Identification of major metabolites of the catechol-*O*-methyltransferase inhibitor entacapone in rats and humans. *Drug Metab. Dispos.* **21**: 81–92