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Dimeric L-Dopa Derivatives as Potential Prodrugs

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Abstract—A series of dimeric derivatives (+)-**1**, and (+)-**2**, and (+)-**3a–d** of L-Dopa diacetyl esters was synthesized and evaluated as potential L-Dopa prodrugs with improved physicochemical properties. All the new compounds showed chemical stability in aqueous buffer solutions (pH 1.3 and 7.4). A relatively slow release of L-Dopa in human plasma was observed.

Parkinson's disease is characterized by a specific loss of dopamine neurons in the substantia nigra (SN). Dopamine deficiency appears to be responsible for the motor deficits of the disorder and at present L-Dopa remains the mainstay of treatment for Parkinson's disease.[1] However, during chronic treatment with L-Dopa, a variety of problems may emerge. Patients experience a decrease in the duration of drug effect ('wearing-off' phenomenon) and, as the number of functioning dopamine neurons decreases in the SN, the patient becomes more sensitive to L-Dopa plasma level fluctuations (on/off effects). L-Dopa is usually administered orally but the drug is extensively metabolized in g.i. tract, so that relatively little arrives in the bloodstream as intact L-Dopa. In addition, it has not been easy to produce a sustained release preparation of L-Dopa capable of more effectively maintaining adequate plasma levels. For this reason, increasing interest has been addressed toward the production of prodrugs with improved pharmacological and pharmacokinetic properties compared with L-Dopa.

Several L-Dopa derivatives were reported with the aim of enhancing its chemical stability, the water or lipid solubility, as well as diminishing the susceptibility to enzymatic degradation.[2–5] Recently, dimeric derivatives have become a common strategy for the production of prodrug forms, in which two identical structural molecules are linked together through a spacer and after administration are metabolized into their identical agents.[6–9]

In this study we have thus protected all the three sensitive centers of L-Dopa: the carboxy function, the amino group and the catechol system, synthesizing a series of dimeric derivatives (+)-**1**, (+)-**2**, and (+)-**3a–d** of diacetyl L-Dopa methyl ester (Fig. 1).

The compounds were evaluated as potential prodrugs with improved physicochemical properties. The present paper reports the lipophilicity and the rates of chemical and enzymatic hydrolysis of all the new compounds.

The amides (+)-**2** and (+)-**3b–d** were synthesized by the classical methods through the interaction of 3,4-diacetyloxy-L-phenylalanine methyl ester hydrochloride

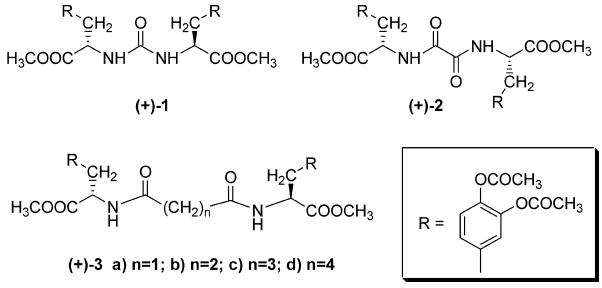

Figure 1.

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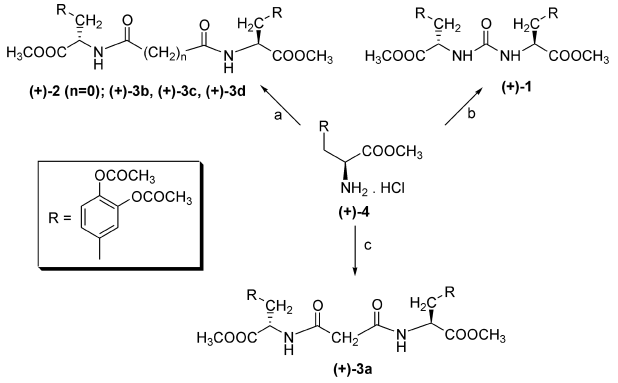
(+)-**4** with the respective dicarboxylic acid dichloride[10] (Scheme 1). The urea (+)-**1** was prepared by the condensation of triphosgene with the derivative (+)-**4** in the presence of Et_3N and the malonamide derivate (+)-**3a** was obtained by treatment of aminoester (+)-**4** with malonic acid and 1,3-dicyclohexylcarbodiimide (DCC).[11]

The apparent partition coefficients (log P) were determined in *n*-octanol/phosphate buffer of pH 7.4. The concentration of prodrugs in the octanol and buffer layer was determined by correlating the peak areas in HPLC to known concentrations of the compounds.

The determined values of log P and physicochemical properties are listed in Table 1. The data show that the lipophilicity increases as the acyl chain is lengthened (see compounds (+)-**3a**–**d**) and the urea (+)-**1** shows



Scheme 1. Reagents: (a) $ClCO(CH_2)_nCOCl$ ($n=0$, 2, 3, 4), Et_3N, THF, 0 °C, then rt, 30 min, 60%; (b) $(CCl_3O)_2CO$, Et_3N, THF, 0 °C, 1 h, 40%; (c) $CH_2(CO_2H)_2$, DCC, Et_3N, CH_2Cl_2, −15 °C, then rt, 30 min, 38%.

the highest value of all our compounds. The solubility of prodrugs was more than the 15 μg/mL necessary for good oral absorption.[12]

The chemical hydrolysis rates of our compounds were measured under two pH conditions (pH 1.3 and 7.4) at 37 °C.[13] The reactivities to chemical hydrolysis were evaluated by pseudo-first-order rate constants obtained from slopes of semilogarithmic plots of the prodrugs concentration against time. The decomposition products were the compounds **5** in which the amide bonds remain unhydrolyzed with loss of ester groups (see Fig. 2).

These derivatives were identified by LC/MS and NMR analysis. The rate constants (K_{obs}) for both chemical hydrolysis and the corresponding half-life times are listed in Table 2.

The rate data in Table 2 show that all the compounds are stable under aqueous buffer solutions of pH 1.3 and pH 7.4.

In 80% rat plasma (rat plasma containing 20% 0.02 M phosphate buffer pH 7.4), catechol esters and amide bonds of the studied derivatives were cleaved and L-DOPA was formed in one step. A rapid conversion of our

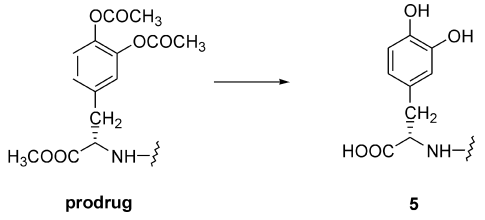


Figure 2.

Table 1. Physicochemical properties of prodrugs (+)-**1**, (+)-**2**, and (+)-**3a**–**d**

Compd	log P[a]	Melting point, °C (recryst. solvent)	$[\alpha]_D^{20}$
(+)-**1**	1.04 (±0.011)	190–192 (MeOH)	+ 61.9° (*c* 1, $CHCl_3$)
(+)-**2**	0.73 (±0.016)	187–189 (EtOH)	+ 61.3° (*c* 1, $CHCl_3$)
(+)-**3a**	0.62 (±0.013)	128–129 (EtOH)	+ 46.2° (*c* 1, $CHCl_3$)
(+)-**3b**	0.85 (±0.013)	162–164 (AcOEt)	+ 69.8° (*c* 1, MeOH)
(+)-**3c**	0.87 (±0.024)	193–195 (MeOH)	+ 23.3° (*c* 1, $CHCl_3$)
(+)-**3d**	0.92 (±0.020)	159–161 (MeOH)	+ 58.5° (*c* 1, $CHCl_3$)

[a]Values are means of three experiments, standard deviation is given in parentheses.

Table 2. Kinetic data for chemical hydrolysis of prodrugs (+)-**1**, (+)-**2**, and (+)-**3a–d** at 37 °C

	pH 1.3[a]		pH 7.4[b]	
Compd	$t_{1/2}$ (h)[c]	K_{obs} (h^{-1})[c]	$t_{1/2}$ (h)[c]	K_{obs} (h^{-1})[c]
(+)-**1**	16.5 (±0.9)	0.042 ($\pm 2.3\times10^{-3}$)	14.2 (±0.7)	0.049 ($\pm 2.5\times10^{-3}$)
(+)-**2**	20.0 (±1.3)	0.032 ($\pm 2.3\times10^{-3}$)	14.2 (±1.3)	0.049 ($\pm 4.2\times10^{-3}$)
(+)-**3a**	16.1 (±1.2)	0.043 ($\pm 3.2\times10^{-3}$)	15.6 (±1.1)	0.040 ($\pm 2.6\times10^{-3}$)
(+)-**3b**	55.4 (±3.1)	0.13 ($\pm 7.2\times10^{-3}$)	16.2 (±4.5)	0.043 ($\pm 1.2\times10^{-3}$)
(+)-**3c**	68.1 (±2.8)	0.010 ($\pm 4.2\times10^{-4}$)	13.9 (±8.1)	0.050 ($\pm 3.1\times10^{-3}$)
(+)-**3d**	48.8 (±1.2)	0.014 ($\pm 3.5\times10^{-4}$)	17.3 (±0.8)	0.040 ($\pm 1.7\times10^{-3}$)

[a]Hydrochloric acid buffer (0.2 M).
[b]Phosphate buffer (0.02 M).
[c]Values are means of three experiments, standard deviation is given in parentheses.

Table 3. Rate constants for the hydrolysis of prodrugs (+)-**1**, (+)-**2**, and (+)-**3a–d** in 80% rat plasma and 80% human plasma at 37 °C

Compd	Rat plasma		Human plasma	
	$t_{1/2}$ (min)[a]	K_{obs} (min^{-1})[a]	k_1 (min^{-1})[a] $t_{1/2}$ (min)[a]	k_2 (min^{-1})[a] $t_{1/2}$ (min)[a]
(+)-**1**	24.4 (±1.9)	0.028 ($\pm 2.2\times10^{-3}$)	0.0068 ($\pm 4\times10^{-4}$) 101.9 (±2.1)	0.0025 ($\pm 1\times10^{-4}$) 277.2 (±8.9)
(+)-**2**	6.1 (±0.21)	0.11 ($\pm 3.8\times10^{-3}$)	0.0083 ($\pm 3\times10^{-4}$) 83.0 (±2.3)	0.0045 ($\pm 3\times10^{-4}$) 152.8 (±7.2)
(+)-**3a**	1.4 (±0.02)	0.43 ($\pm 6.2\times10^{-3}$)	0.072 ($\pm 4\times10^{-3}$) 9.0 (±0.4)	0.0052 ($\pm 2\times10^{-4}$) 133.27 (±6.5)
(+)-**3b**	2.0 (±0.08)	0.34 ($\pm 1.4\times10^{-2}$)	0.050 ($\pm 3\times10^{-3}$) 13.9 (±0.5)	0.0086 ($\pm 5\times10^{-4}$) 50.6 (±2.7)
(+)-**3c**	2.9 (±0.11)	0.24 ($\pm 9.1\times10^{-3}$)	0.011 ($\pm 2\times10^{-3}$) 63.0 (±2.9)	0.0045 ($\pm 1\times10^{-4}$) 154.0 (±10.2)
(+)-**3d**	4.1 (±0.17)	0.17 ($\pm 7.0\times10^{-3}$)	0.081 ($\pm 2\times10^{-3}$) 8.0 (±0.2)	0.010 ($\pm 2\times10^{-3}$) 73.3 (±3.1)

[a]Values are means of three experiments, standard deviation is given in parentheses.

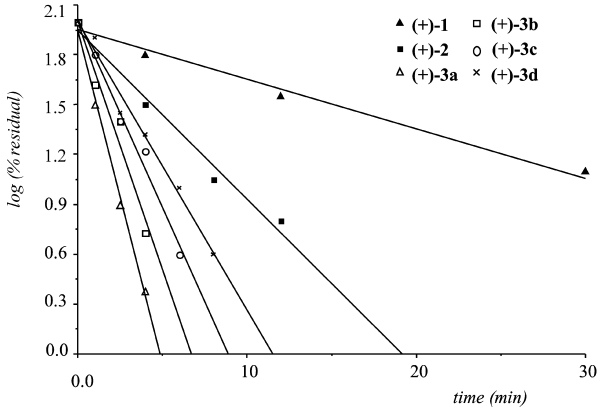


Figure 3. First-order kinetics for hydrolysis of prodrugs (+)-**1**, (+)-**2**, and (+)-**3a–d** in rat plasma diluted to 80% (v/v) with pH 7.4 isotonic phosphate buffer.

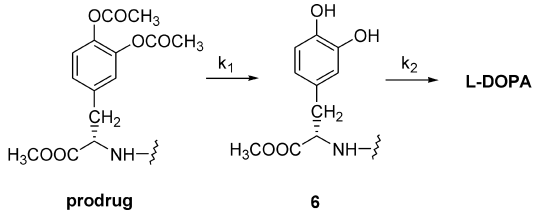


Figure 4.

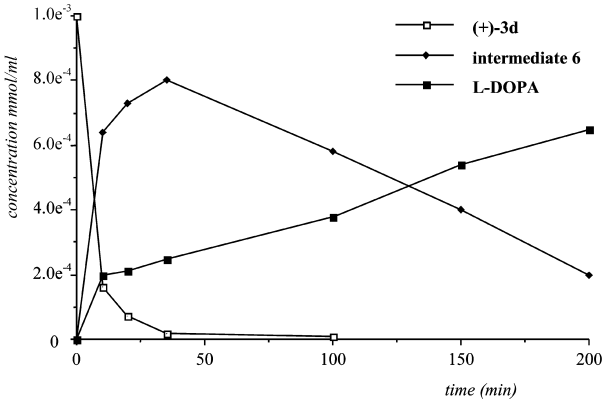


Figure 5. Time courses for hydrolysis of prodrug (+)-**3d** to L-DOPA via the intermediate formation of **6** in human plasma diluted to 80% (v/v) with pH 7.4 isotonic phosphate buffer.

compounds to L-DOPA was observed; the data are illustrated in Figure 3 and listed in Table 3.

Hydrolysis in 80% human plasma (human plasma containing 20% 0.02 M phosphate buffer pH 7.4), proceeds slowly by a two-step reaction: at first the ester groups are cleaved with formation of intermediate **6** which, afterwards, is hydrolyzed to L-Dopa (Fig. 4). The rate constants (K_{obs}) and the corresponding half-life times are shown in Table 3.

The degradation process was found correlated with first-order kinetics and the L-Dopa was released in quantitative amounts.

An example of the time course for enzymatic hydrolysis observed in 80% human plasma is illustrated in Fig. 5. All the compounds are converted to L-Dopa after enzymatic hydrolysis and they showed a sustained release of the parent drug in human plasma. The acyl spacer has little influence on the rates of prodrug bioconversion.

In conclusion, dimeric L-Dopa derivatives were synthesized. An HPLC and LC/MS was used for investigation of the hydrolysis kinetics in aqueous buffer solution and in 80% rat and human plasma. The lipophilicity parameters (log P) were determined and seem sufficient for good oral absorption after oral administration. A considerable chemical stability of all prodrugs was observed at 37 °C in aqueous buffer solutions of pH 1.3 (non-enzymatic Simulated Gastric Fluid, SGF) and isotonic phosphate buffer of pH 7.4. A relatively slow release of L-Dopa in 80% human plasma was observed while in 80% rat plasma all the prodrugs were rapidly cleaved into L-Dopa.[14]

Microdialysis studies in rat striatum are in progress in order to evaluate the bioavailability after oral and parenteral administration.

Acknowledgements

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11. The purity of all new compounds was checked by HPLC using the column Merck Purospher RP-18 endcapped (5 μm) 125–3 with MeOH/H_2O 60:40 as eluent. The microanalyses results were within ±0.4%. ^{1}H NMR spectra were recorded at 300 MHz in DMSO-d_6 as solvent. Compound (+)-**1**: δ ppm 7.24–7.04 (m, 6H, ArH), 6.67–6.56 (d, 2H, 2NH), 4.46–4.32 (m, 2H, 2CH-N), 3.61 (s, 6H, $2OCH_3$), 3.08–2.82 (m, 4H, $2CH_2$-Ar), 2.27 (s, 12H, $4CH_3CO$). Compound (+)-**2**: δ ppm 9.20–9.07 (d, 2H, 2NH), 7.15 (s, 6H, ArH), 4.61–4.45 (m, 2H, 2CH-N), 3.61 (s, 6H, $2OCH_3$), 3.20–3.04 (m, 4H, $2CH_2$-Ar), 2.25 (s, 12H, $4CH_3CO$). Compound (+)-**3a**: δ ppm 8.60–8.50 (d, 2H, 2NH), 7.20–7.10 (m, 6H, ArH), 4.55–4.40 (m, 2H, 2CH-N), 3.58 (s, 6H, $2OCH_3$), 3.12 (s, 2H, CH_2CO), 3.04–2.92 (m, 4H, $2CH_2$-Ar), 2.26 (s, 12H, $4CH_3CO$). Compound (+)-**4**: δ ppm 8.74 (s, 3H, NH_3^+), 7.30–7.18 (m, 3H, ArH), 4.36–4.22 (m, 1H, CH-N), 3.68 (s, 3H, OCH_3), 3.50–3.22 (m, 2H, CH_2-Ar), 2.29 (s, 6H, $2CH_3CO$). The optical purity of all the new compounds was established after hydrolysis with HCl 37%/EtOH 1:1 (refluxed for 3 h). The recovered L-Dopa showed at least an e.e. >96%.
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