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Original article

Design, synthesis and biological evaluation of L-dopa amide derivatives as potential prodrugs for the treatment of Parkinson's disease

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ABSTRACT

A range of amide derivatives of L-dopa were synthesized and investigated for their pharmacological activity and their ability to be converted to L-dopa using the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rat, as an experimental model of Parkinson's disease. The diacetyl derivative of L-dopa amide (**11b**) was found to be more active than L-dopa after its oral administration and generated plasma levels of L-dopa in the therapeutic range for an antiparkinsonian effect in man.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder resulting in motor disability, primarily associated with progressive degeneration of pigmented neurones in the substantia nigra pars compacta, resulting in a reduction of striatal dopamine levels and the formation of intracellular proteinous inclusions, termed Lewy bodies mainly composed of fibrillar α-synuclein [1,2]. Dopamine replacement therapy using L-dopa or dopamine agonist drugs, such as ropinirole and pramipexole, is the mainstay of treatment resulting in an initial normalisation of motor function [3,4]. L-dopa is the most effective of these medications and all patients require its use at some point in the course of the illness. However, on chronic use and with disease progression, the effects of L-dopa become shorter (wearing-off), unpredictable (on-off) and involuntary movements termed dyskinesia can appear [5,6]. Wearing off and dyskinesia are in part due to the erratic absorption of L-dopa from the gut and peaks and troughs in its plasma levels that leads to pulsatile stimulation of striatal post-synaptic dopamine receptors [7]. This coupled to the rapid metabolism of L-dopa and its short plasma half-life causes a non-physiological response in the basal ganglia that causes the motor complications that make the later treatment of PD complex.

Numerous attempts have been made to overcome the problem of erratic absorbtion, rapid metabolism and short half-life, among which prodrug design has been the most promising [8]. Recent examples have included amide derivatives linked to glutathione or other antioxidants but where the objective was to achieve a symptomatic effect in PD associated with modification of the disease progression through inhibition of oxidative stress [9,10]. These derivatives did not, however, lead to the release of L-dopa in a manner that was more prolonged than that achieved by L-dopa itself. Indeed, simple amide derivatives made hold more promise and recently activity was reported with a class of L-dopamides [11]. But to date, there has been no detailed investigation of the structure-activity relationship of L-dopa amide derivatives. We report herein the pharmacological and pharmacokinetic behaviour of a series of L-dopamide prodrugs in the 6-hydroxydopamine (OHDA)-lesioned rat that acts as a functional model of drug effect in PD [12].

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2. Results

2.1. Chemistry

In order to investigate the inference of different substitutents on the amide and catechol functions, on the pharmacological properties on L-dopamide, we have designed and synthesized a series of L-dopamide derivatives (**9a-9e**, **10b-10g**, **11b-11f**). A range of protected amide derivatives of L-dopa (**6a-6g**) were synthesized as shown in Scheme 1. The methyl ester **2** was obtained by reaction of L-dopa with thionyl chloride in methanol. The protection of the amino group of **2** was achieved by treating **2** with either di-*tert*-butyl-dicarbonate or benzyl chloroformate to obtain **3a** or **3b**. Further protection of the hydroxyl groups on **3a** and **3b** was achieved by treatment with benzyl bromide in the presence of potassium carbonate resulting in **4a** and **4b**, which generated acids **5a** and **5b**, respectively, on hydrolysis in lithium hydroxide. Coupling of **5a** or **5b** with various amines was carried out in the presence of N-hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) at room temperature, providing a range of protected amide derivatives of L-dopa (**6a**–**6g**). The advantages of using the coupling conditions in this reaction are high yield, simplicity of manipulation and ease of product purification.

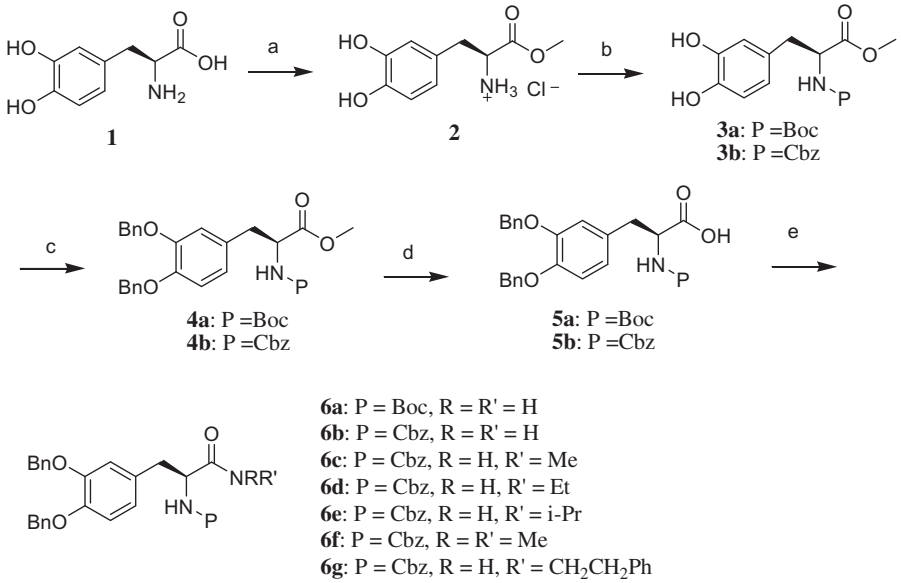
As L-3-(3-hydroxy-4-pivaloyloxyphenyl)alanine produces a sustained L-dopa plasma level and large L-dopa bioavailability after oral dosing in rats and dogs [13], the pivaloyl group and several other groups possessing similar lipophilicity were employed to protect the hydroxyl groups on catechol. The synthesis of 3,4-diacyl L-dopa amide is presented in Scheme 2. Debenzylation of **6a** was achieved by hydrogenation in the presence of Pd/C to generate **7**. Acylation of catechol hydroxyl functions on **7** was carried out by treatment with a series of acyl chlorides to form **8a**–**8e**, which when subjected to deprotection of the Boc group in hydrogen chloride solution provided the final products **9a**–**9e**.

Hydrogenation of **6b**–**6g** in the presence of Pd/C and hydrochloric acid provided the hydrochloride salts of the L-dopa amide derivatives **10b**–**10g**. Diacetylation of **10b**–**10f** was carried out by treatment with acetyl chloride in the presence of hydrogen chloride to produce **11b**–**11f**, respectively (Scheme 3). This step was used for acetyl group as it is sensitive to strong acid conditions. When Boc-protected diacetyl L-dopa amide is treated with hydrochloric acid in dioxane, the acetyl groups are partially hydrolysed, leading to a product of low purity.

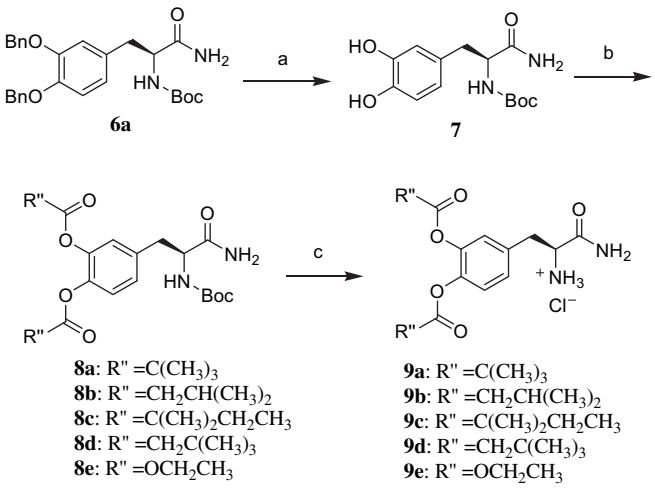
Conversion of the carboxylic acid function of L-dopa to the simple amide (**10b**) led to a marked increase in the clogP value. The clogP value is a useful measure of the ability of a compound to penetrate biological membranes by simple diffusion. LogP is the log of the partition coefficient of a non-charged compound between n-octanol and aqueous phase and has been widely adopted as one of a number of important parameters which can lead to the prediction of membrane permeability [14]. Thus the marked increase of the clogP value between L-dopa and the simple amide **10b** would suggest improved membrane permeability of the latter molecule. As the size of the alkyl substituent on the amide function increase there is a parallel increase in the clogP value [15] (Table 1). A surprising feature of this study is that in every case the conversion of both the catechol hydroxyl functions to acetyl groups leads to an increase in hydrophilicity (that is a decrease in clogP value (Table 1). Thus the introduction of the two acetyl functions is unlikely to enhance the membrane penetration of these molecules by non facilitated transport.

2.2. Pharmacological evaluation

The potential anti-parkinsonian activity of L-dopa and the prodrugs was assessed in the unilaterally 6-OHDA-lesioned rat where the administration of L-dopa leads to a marked contraversive rotational response. L-Dopa and the L-dopamides **10b**, **10c**, **10d**, **10g** were administered both orally and intraperitoneally (ip). In 6-OHDA-lesioned rats, **10b** was found to be the most active, with oral administration being less effective than ip administration. Activity was more pronounced than seen with L-dopa but was of similar duration. **10c** had very low activity when administered by both routes and was of short duration, and **10d** possessed moderate activity but this was less intense and of shorter duration than seen with L-dopa. In contrast good activity was associated with the ip administration of **10g**, in fact much higher activity than experienced following oral administration of **10g** but the response was not of longer duration than occurred with L-dopa.



Scheme 1. Reagents and conditions: (a) MeOH, $SOCl_2$, −5 °C then reflux; (b) $(Boc)_2O$, or CbzCl, DMF, 0 °C; (c) BnBr, K_2CO_3, acetone; (d) LiOH, THF/MeOH; (e) i) NHS, EDC, ii) RR′NH.



Scheme 2. Reagents and conditions: (a) 30 psi H_2, Pd/C, EtOAc/MeOH; (b) R″COCl, Et_3N, CH_2Cl_2, 0 °C; (c) HCl-dioxane, CH_2Cl_2.

In general there was lower activity observed with acylated catechol derivatives both in terms of the overall rotational response and the duration of effect, with the exception of **11b** which was active when administered by either the oral or intraperitoneal routes and showed a response that was equivalent to or greater than that produced by L-dopa. **11c**–**11f** were all found to be largely devoid of activity. The dicarbonate derivative of **10b** (**9e**) was found to be active, although with a lower activity than that of the corresponding diacetyl derivative (Table 1).

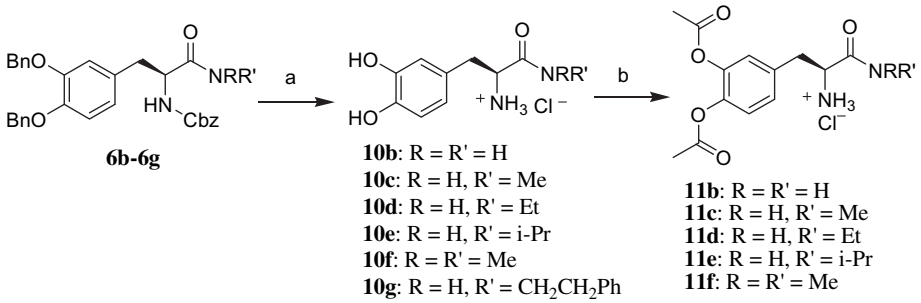
Although L-dopa itself is a prodrug of dopamine, it is generally accepted that the therapeutic effects of L-dopa prodrugs can be correlated with the blood L-dopa concentration resulting from cleavage of the prodrug, as the amount of dopamine generated in the brain depends on the amount of L-dopa crossing the blood brain barrier. Thus, we determined the L-dopa concentration in the blood at various times after administration of equivalent doses of some of the compounds. The results are summarised in Table 2. **10b** and **11b** gave the largest AUC and C_{max} values but only **11b** had an extended $t_{1/2}$ compared to L-dopa. The compounds with the more hydrophobic ester protecting groups (**9a**, **9b** and **9e**) gave rise to less L-dopa than the corresponding acetyl compound (**11b**) (Table 2).

3. Discussion

6-OHDA-lesioned rat model has been extensively used to study dopamine neurons in the brain, to investigate the role of these dopamine neurons with respect to behaviour, to examine the brain's capacity to recover from or compensate for specific neurochemical depletions, and to investigate the promotive effects of experimental and clinical approaches which are relevant for the treatment of Parkinson's disease.[12] The injection of 6-OHDA selectively damage mesencephalic dopamine neurons on one side of the brain, causing the reduce of dopamine at the ipsilateral side as the injection side of 6-OHDA. This leads to ipsiversive turning. The dopamine receptor agonist apomorphine can induce asymmetries in turning. This contraversive response is attributed to the stimulation of supersensitive dopamine receptor mechanisms, especially at the level of the dopamine-depleted neostriatum. In animals with unilateral 6-OHDA lesions, the behavioural effects of L-dopa are similar to those of the unselective dopamine receptor agonist apomorphine, as L-dopa generates dopamine and compensates the loss of dopamine at the damaged side after it enters brain. Thus, the systemic administration of L-dopa acutely induces intense contraversive turning[16].

The results show that some of the dopamide derivatives synthesized in this study are converted to L-dopa in the 6-OHDA lesioned rat in sufficient quantity to produce a behavioural response. This is significant as this experimental model of PD is highly predictive of drug effect in man. Compound **11b** in particular showed activity after oral administration that resulted in a greater pharmacological response and prolonged plasma levels of L-dopa compared to a molar equivalent dose of L-dopa.

It is clear that the acetyl and unsubstituted amide functions were rapidly cleaved, leading to the appearance of L-dopa in plasma. As there was a high yield of L-dopa generated when **10b** was administered *via* the ip route there must be high peptidase activity in the serum, which is capable of cleaving the unsubstituted amide. In contrast, only low levels of L-dopa resulted when **10c** and **10d** were administered via ip route. This difference is probably linked with the inability of the N-alkylated prodrugs to be cleaved in the plasma [17,18]. As **11b** produces a prolonged plasma $t_{½}$ for L-dopa compared to **10b** when both are administered via the oral route, there appears to be a beneficial effect in acetylating both the phenolic functions. Presumably this is associated with the increased ability of **11b** to permeate the gut and to enter the general circulation. Although double acetylation fails to increase the clogP value (Table 1), the decreased hydrogen bonding potential of the diesters, when compared with the H-donating catechol function is probably responsible for the enhanced absorption of this molecule. As the pKa value of the α-amine function is 8.9, there will always be an appreciable portion of the neutral form of **11b** present in the lumen of the intestine. The acetyl ester functions have been demonstrated to be stable at both acid and neutral pH values (data not presented) and are therefore likely to survive exposure to the lumen of the stomach and small intestine, whereas in the serum the diacetyl derivative (**11b**) is rapidly converted to L-dopa. With the exception of **11b**, none of the other diacetyl prodrugs investigated in this study led to appreciable blood levels of L-dopa after oral administration. This confirms the suggestion that the N-alkylated amide prodrugs are not cleaved in plasma, even though they are predicted to be absorbed from the gut.



Scheme 3. Reagents and conditions: (a) 30 psi H_2, Pd/C, HCl, EtOAc/MeOH; (b) HOAc-HCl, AcCl.

Table 1
PD analysis.

Prodrugs	Route of administration	Dose		AUC activity (rot/test period)	Maximum activity (rot/10 min)	Duration of activity (min)	clogP
		(mg/kg)	(μmol/kg)				
L-Dopa	p.o.	12.5	63.39	522	71	119	−2.20
L-Dopa	i.p.	12.5	63.39	1021	104	160	
10b	p.o.	14.74	63.35	612	105	103	−0.70
10b	i.p.	14.74	63.35	1186	144	155	
10c	p.o.	15.64	63.66	29	5	10	−0.88
10c	i.p.	15.64	63.66	16	2	0	
10d	p.o.	16.53	63.65	324	39	39	−0.51
10d	i.p.	16.53	63.65	117	40	18	
10g	p.o.	21.35	63.58	94	17	30	0.92
10g	i.p.	21.35	63.58	825	124	124	
11b	p.o.	20.1	63.46	1085	107	147	−1.09
11b	i.p.	20.1	63.46	838	69	140	
11c	p.o.	20.97	63.40	26	1	0	−1.28
11d	p.o.	21.86	63.40	20	6	3	−0.90
11e	p.o.	22.75	63.40	26	2	0	−0.60
11f	p.o.	21.81	63.26	26	1	0	−1.47
9e	p.o.	23.89	63.40	579	90	153	0.37

Vehicle: saline; test period: 240 min.

It is assumed on the basis of PD analysis that **10g** did not generate high levels of blood L-dopa when administered orally but did so when administered by the ip route. The reason for the poor absorption from the intestine is not clear, particularly as **10g** has a relatively high logP value. Generation of high levels of L-dopa of this molecule, when administered via the ip route, indicates that there is a serum peptidase capable of cleaving the aryl substituted amide function.

Although the dicarbonate (**9e**) results in high L-dopa levels, it is not as efficient as the corresponding diacetyl derivative (**11b**). Introduction of a more hydrophobic ester group on the catechol function reduces the rate of conversion to L-dopa in the serum.

The most efficient prodrug of the present series for producing more prolonged blood levels of L-dopa, when administered via the oral route, is the diacetyl derivative of the simple L-dopa amide (**11b**). After oral administration, this molecule resulted in a rotational response that was double that seen after administration of L-dopa with a greater maximal effect and a longer duration of action. The characteristics of the compound are favourable when compared to the absorption of orally administered L-dopa, which in the same experimental system is about 50% of that corresponding to **11b** (Table 1). The resulting levels of L-dopa in plasma at C_{max} are in the therapeutic range known to result in an antiparkinsonian response in man.

4. Experimental

4.1. General

All chemicals were purchased from Aldrich or Fluka and were used without further purification. ^{1}H NMR Spectra were recorded on Bruker Avance 400 spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent with TMS as an internal standard. Electrospray ionization (ESI) mass spectra were obtained by infusing samples into an LCQ Deca XP ion trap instrument. High resolution mass spectra (HRMS) were obtained on a QTOF Micro by direct infusing samples into the EI source.

4.1.1. Preparation of (S)-3-(3,4-dihydroxyphenyl)-1-methoxy-1-oxopropan-2-aminium chloride (**2**)

To methanol (50 mL) cooled to −5 °C was added thionyl chloride (5 mL) slowly and the temperature was kept under 10 °C. L-dopa (10 g, 50.7 mmol) was then added in portions. The solution was slowly warmed to room temperature and then stirred at 50 °C overnight. After removal of the solvent, the residue was redissolved in methanol, and titratated with diethyl ether, and stored at 4 °C. The product **2** was obtained as a white solid by filtration, 11.6 g (92% yield). ^{1}H NMR (DMSO-d_6) δ 2.90–3.03 (m, 2H), 3.64 (s, 3H), 4.10 (m, 1H), 6.45 (m, 1H), 6.61 (m, 1H), 6.69 (m, 1H), 8.61 (br, 3H), 8.97 (s, 1H), 9.00 (s, 1H). ESI-MS: m/z 212 ([M−Cl]$^+$).

4.1.2. Preparation of (S)-methyl 2-(tert-butoxycarbonyl)-3-(3,4-dihydroxyphenyl)propanoate (**3a**)

To a solution of **2** (11 g, 44.4 mmol) in THF (80 mL) was added saturated aqueous $NaHCO_3$ (80 mL). The resulting solution was cooled with ice-bath and di-*tert*-butyl dicarbonate (10.66 g in 50 mL THF, 48.84 mmol) was added dropwise. The reactant was allowed to warm to room temperature and stirred for 1 h. The organic solvent was removed and the aqueous layer was extracted with dichloromethane (2 × 60 mL). The combined organic layers were washed subsequently with water, aqueous $KHSO_4$, brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel chromatography using ethyl acetate/hexane (1:1) as an eluent to afford product **3a** as a white solid (13.0 g, 94%). ^{1}H NMR (DMSO-d_6) δ 1.34 (s, 9H), 2.63–2.80 (m, 2H), 3.59(s, 3H), 4.04 (m, 1H), 6.45 (m, 1H), 6.60 (m, 2H), 7.18 (d, J = 8.0 Hz, 1H, NH), 8.75 (br, 2H, OH). ESI-MS: m/z 312 (MH$^+$).

Table 2
Pharmacokinetic analysis result.

Compound	Route of admin.	Dose (mg/kg)	Dose (μmol/kg)	L-dopa Cmax (ng/ml)	L-dopa $t_{1/2}$ apparent (min)	L-dopa AUC (0–4 h) (μg.min/ml)
L-dopa	p.o.	12.5	63.5	4088	75	434
10b	p.o.	14.8	63.5	2203	52	256
11b	p.o.	20.1	63.5	1746	97	245
9a	p.o.	42.5	106	415	51	44
9b	p.o.	25.4	63.5	860	78	98
9e	p.o.	23.9	63.5	690	ND	95

*4.1.3. Preparation of (S)-methyl 2-(benzyloxycarbonyl)-3-(3,4-dihydroxyphenyl)propanoate (**3b**)*

To a solution of **2** (15.6 g, 63.0 mmol) in anhydrous DMF (100 mL) was added Et_3N (14.0 g, 138.6 mmol). The solution was cooled on an ice-bath and benzyl chloroformate (11.8 g, 69.3 mmol) was added in three portions, stirring was continued at 0 °C for 3 h. Cold aqueous HCl (500 mL, 1.5 M) was added to the reactant and the resulting solution was extracted with diethyl ether (2 × 400 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent, the crude product **3b** was obtained as a colorless oil (21.2 g, 97%) which was used in next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 2.96 (m, 2H, CH_2), 3.71 (s, 3H, OCH_3), 4.60 (m, 1H, CH), 5.09 (s, 2H, CH_2), 5.27 (m, 1H, NH), 6.49 (d, J = 8.0, 1H, 6-ArH), 5.93 (br, 2H, OH), 6.59(s, 1H, 2-ArH), 6.72 (d, J = 8.0 Hz, 1H, 5-ArH), 7.29–7.42 (m, 5H, PhH). ESI-MS: m/z 346 (MH^+).

4.1.4. General procedure for dibenzylation of catechol hydroxyl groups

A mixture of **3a** or **3b** (27 mmol), K_2CO_3 (11.2 g, 81 mmol) and benzyl bromide (13.85 g, 81 mmol) in acetone (200 mL) was refluxed under nitrogen for 3 days, cooled and filtered. The filtrate was concentrated and the residue was then dissolved in dichloromethane (100 mL). The solution was washed with water (2 × 100 mL), brine (100 mL), dried over Na_2SO_4, filtered and concentrated. The residue was washed with cold methanol to afford **4a** or **4b** as white solids.

*4.1.4.1. (S)-methyl 3-(3,4-bis(benzyloxy)phenyl)-2-(tert-butoxycarbonyl) propanoate **(4a)**.* Yield 92%; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, Boc-CH_3), 2.98 (m, 2H, CH_2), 3.64 (s, 3H, OCH_3), 4.52 (m, 1H, CH), 4.94 (d, J = 8.2 Hz, 1H, NH), 5.121 and 5.125 (s, 4H, CH_2), 6.64 (d, J = 8.1 Hz, 1H, 6-ArH), 6.73(d, J = 1.8 Hz, 1H, 2-ArH), 6.85(d, J = 8.1 Hz, 1H, 5-ArH), 7.29–7.45 (m, 10H, PhH). ESI-MS: m/z 492 (MH^+).

*4.1.4.2. (S)-methyl 3-(3,4-bis(benzyloxy)phenyl)-2-(benzyloxycarbonyl) propanoate **(4b)**.* Yield 87%; ^{1}H NMR ($CDCl_3$) δ 3.00 (m, 2H, CH_2), 3.64 (s, 3H, OCH_3), 4.60 (m, 1H, CH), 5.08, 5.10 and 5.12 (s, 6H, CH_2), 5.16 (d, J = 8.2 Hz, 1H, NH), 6.60 (dd, J = 8.2 and 1.9 Hz, 1H, 6-ArH), 6.69(s, 1H, 2-ArH), 6.83(d, J = 8.2 Hz, 1H, 5-ArH), 7.28–7.44 (m, 15H, PhH). ESI-MS: m/z 526 (MH^+).

*4.1.5. General procedure for hydrolysis of **4a** and **4b***

To a solution of **4a** or **4b** (23.76 mmol) in THF/MeOH (3:1, 160 mL) was added aqueous LiOH (2 M, 35.6 mL). The solution was stirred at room temperature for 3 h and then water (300 mL) was added. The resulting solution was acidified to pH 2–3 with aqueous $KHSO_4$ and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic extracts were washed with water, brine, dried over Na_2SO_4. After removal of the solvent, the product was obtained as a white solid.

*4.1.5.1. (S)-3-(3,4-bis(benzyloxy)phenyl)-2-(tert-butoxycarbonyl) propanoic acid **(5a)**.* Yield 97%; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, Boc-CH_3), 2.97-3.05 (m, 2H, CH_2), 4.52 (m, 1H, CH), 4.90 (d, J = 7.3 Hz, 1H, NH), 5.11 (s, 4H, CH_2), 6.68 (d, J = 8.1 Hz, 1H, 6-ArH), 6.78(s, 1H, 2-ArH), 6.85(d, J = 8.1 Hz, 1H, 5-ArH), 7.29–7.44 (m, 10H, PhH). ESI-MS: m/z 478 (MH^+).

*4.1.5.2. (S)-3-(3,4-bis(benzyloxy)phenyl)-2-(benzyloxycarbonyl) propanoic acid **(5b)**.* Yield 98%; ^{1}H NMR ($CDCl_3$) δ 2.95-3.09 (m, 2H, CH_2), 4.60 (m, 1H, CH), 5.05 (1H, NH, buried), 5.08 and 5.10 (s, 6H, CH_2), 6.63 (dd, J = 8.1 and 1.8 Hz, 1H, 6-ArH), 6.72(s, 1H, 2-ArH), 6.81 (d, J = 8.1 Hz, 1H, 5-ArH), 7.29–7.42 (m, 15H, PhH). ESI-MS: m/z 512 (MH^+).

*4.1.6. General preparation of dibenzyl protected L-dopa amide derivatives **6a–6g***

A mixture of **5a** or **5b** (10.26 mmol), NHS (1.42g, 12.32 mmol), EDC (2.36g, 12.32 mmol) and CH_2Cl_2/DMF (3:1, 140 mL) was stirred at room temperature overnight. NH_3 solution (0.5 M in dioxane, 24.65 mL) or other amine (1.2 equiv) was then added with continued stirring for 4 h. The reactant was diluted with $CHCl_3$, and washed with saturated $NaHCO_3$, brine, and dried over Na_2SO_4. After removal of the solvent, the residue was washed with cold methanol/diethyl ether to afford product as a white solid.

*4.1.6.1. (S)-tert-butyl 3-(3,4-bis(benzyloxy)phenyl)-1-amino-1-oxopropan-2-ylcarbamate **(6a)**.* Yield 96%; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, Boc-CH_3), 2.84–2.89 and 3.01–3.06 (m, 2H, CH_2), 4.23 (m, 1H), 5.08 (m, 2H, NH_2), 5.14 and 5.16 (s, 4H, CH_2), 5.48 (br, 1H, NH), 6.72 (d, J = 8.1, 1H, 6-ArH), 6.81 (s, 1H, 2-ArH), 6.86 (d, J = 8.1 Hz, 1H, 5-ArH), 7.28–7.45 (m, 10H, PhH). ESI-MS: m/z 477 (MH^+).

*4.1.6.2. (S)-benzyl 3-(3,4-bis(benzyloxy)phenyl)-1-amino-1-oxopropan-2-ylcarbamate **(6b)**.* Yield 93%; ^{1}H NMR ($CDCl_3$) δ 2.82–2.88 and 3.05–3.09 (m, 2H, CH_2), 4.28 (m, 1H, CH), 5.08 (br, 1H, NH), 5.09 and 5.14 (s, 6H, CH_2), 5.32 (m, 2H, NH_2), 6.70 (d, J = 8.0 Hz, 1H, 6-ArH), 6.79(s, 1H, 2-ArH), 6.85 (d, J = 8.1 Hz, 1H, 5-ArH), 7.29–7.45 (m, 15H, PhH). ESI-MS: m/z 511 ($[M+H]^+$), 533 ($[M+Na]^+$).

*4.1.6.3. (S)-benzyl 3-(3,4-bis(benzyloxy)phenyl)-1-(methylamino)-1-oxopropan-2-ylcarbamate **(6c)**.* Yield 93%; ^{1}H NMR ($CDCl_3$) δ 2.60 (d, J = 4.9 Hz, 3H, CH_3), 2.82–2.88 and 3.02–3.07 (m, 2H, CH_2), 4.22 (m, 1H, CH), 5.08 and 5.13 (s, 6H, CH_2), 5.32 (m, 1H, NH), 5.40(m, 1H, NH), 6.65 (d, J = 7.9 Hz, 1H, 6-ArH), 6.79(s, 1H, 2-ArH), 6.83 (d, J = 8.1 Hz, 1H, 5-ArH), 7.28–7.44 (m, 15H, PhH). ESI-MS: m/z 525 ($[M+H]^+$), 547 ($[M+Na]^+$).

*4.1.6.4. (S)-benzyl 3-(3,4-bis(benzyloxy)phenyl)-1-(ethylamino)-1-oxopropan-2-ylcarbamate **(6d)**.* Yield 90%; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.2 Hz, 3H, CH_3), 2.79–2.85 (m, 1H, DOPA-CH_2), 3.03–3.16 (m, 3H, 2H from CH_2 and 1H from DOPA-CH_2), 4.18 (m, 1H, CH), 5.10 and 5.13 (s, 6H, CH_2), 5.24 (m, 1H, NH), 5.34(m, 1H, NH), 6.66 (d, J = 8.2 Hz, 1H, 6-ArH), 6.83 (m, 2H, 2-ArH and 5-ArH), 7.28–7.44 (m, 15H, PhH). ESI-MS: m/z 539 ($[M+H]^+$), 561($[M+Na]^+$).

*4.1.6.5. (S)-benzyl 3-(3,4-bis(benzyloxy)phenyl)-1-(isopropylamino)-1-oxopropan-2-ylcarbamate **(6e)**.* Yield 85%; ^{1}H NMR ($CDCl_3$) δ 0.84 (d, J = 6.5 Hz, 3H, CH_3), 0.98 (d, J = 6.5 Hz, 3H, CH_3), 2.77–2.83 and 3.03–3.09 (m, 2H, CH_2), 3.84–3.93(m, 1H, CH), 4.15 (m, 1H, CH), 5.06 (d, J = 8.0 Hz, 1H, NH), 5.10 and 5.13 (s, 6H, CH_2), 5.39(m, 1H, NH), 6.66 (d, J = 7.9 Hz, 1H, 6-ArH), 6.83 (d, J = 8.1 Hz, 2H, 2-ArH and 5-ArH), 7.27–7.43 (m, 15H, PhH). ESI-MS: m/z 553 ($[M+H]^+$), 575($[M+Na]^+$).

*4.1.6.6. (S)-benzyl 3-(3,4-bis(benzyloxy)phenyl)-1-(dimethylamino)-1-oxopropan-2-ylcarbamate **(6f)**.* Yield 94%; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 2.76(s, 3H, CH_3), 2.79-2.85 and 2.91-2.96 (m, 2H, CH_2), 4.77 (m, 1H, CH), 5.08 (d, J = 2.1 Hz, 2H, CH_2), 5.10 and 5.13 (s, 4H, CH_2), 5.66 (d, J = 8.6 Hz, 1H, NH), 6.67 (dd, J = 8.1 and 1.8 Hz, 1H, 6-ArH), 6.80 (d, J = 1.8 Hz, 1H, 2-ArH), 6.83 (d, J = 8.1 Hz, 1H, 5-ArH), 7.29–7.46 (m, 15H, PhH). ESI-MS: m/z 539 ($[M+H]^+$), 561 ($[M+Na]^+$).

*4.1.6.7. (S)-benzyl 3-(3,4-bis(benzyloxy)phenyl)-1-oxo-1-(phenethylamino)propan-2-ylcarbamate **(6g)**.* Yield 91%; ^{1}H NMR ($CDCl_3$) δ 2.49–2.67 (m, 2H, CH_2), 2.84 and 3.01 (m, 2H, DOPA-CH_2), 3.26–3.41 (m, 2H, CH_2), 4.18 (m, 1H, DOPA-CH), 5.08, 5.11 and 5.12 (s, 6H, CH_2), 5.28 (m, 1H, NH), 5.46 (t, J = 5.8 Hz, 1H, NH), 6.64 (d, J = 7.8 Hz, 1H, 6-ArH), 6.81 (m, 2H, 2-ArH and 5-ArH), 7.02 (m, 2H, PhH), 7.17–7.43 (m, 18H, PhH). ESI-MS: m/z 615 (MH^+).

*4.1.7. Preparation of (S)-tert-butyl 1-amino-3-(3,4-dihydroxyphenyl)-1-oxopropan-2-ylcarbamate (**7**)*

To a suspension of **6a** (2.38 g, 5 mmol) in MeOH/EtOAc (1:1, 40 mL) was added 5% Pd/C (0.24 g). Hydrogenation was carried out at 30 psi H_2 for 3 h. After filtration to remove the catalyst, the filtrate was concentrated and **7** was obtained as a white solid (1.48 g, 100%). ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 9H, CH_3), 2.55 and 2.76 (m, 2H, DOPA-CH_2), 3.98 (m, 1H, DOPA-CH), 6.49 (d, J = 6.5, 1H, 6-ArH), 6.62 (m, 2H, 2-ArH and 5-ArH), 6.95 and 7.29(s, 2H, NH_2), 8.66 (d, J = 6.8, 1H, NH). ESI-MS: m/z 297 (MH^+).

*4.1.8. General preparation of diacyl Boc-protected L-dopa amide derivatives (**8a**–**8e**)*

To a suspension of **7** (0.5 g, 1.69 mmol) in CH_2Cl_2 (20 mL) cooled on an ice-bath was added Et_3N (0.41 g, 4.06 mmol), followed by the dropwise addition of a variety of acyl chlorides (4.056 mmol). The mixture was stirred for 3 h and then washed with aqueous $NaHCO_3$, brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel chromatography (EtOAc/Hexane 1:1) to obtain the products as white solids.

*4.1.8.1. (S)-tert-butyl 1-amino-3-(3,4-bis(pivaloyloxy)phenyl)-1-oxopropan-2-ylcarbamate **(8a)**.* Yield 62%; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 18H, CH_3), 1.44 (s, 9H, CH_3), 2.95 and 3.14 (m, 2H, CH_2), 4.32 (m, 1H, CH), 5.22 (m, 1H, NH), 5.41 and 5.87 (br, 2H, NH_2), 7.00–7.12 (m, 3H, ArH). ESI-MS: m/z 465 (MH^+).

*4.1.8.2. (S)-tert-butyl 1-amino-3-(3,4-bis(3-methylbutanoyloxy)phenyl)-1-oxopropan-2-ylcarbamate **(8b)**.* Yield 61%; ^{1}H NMR ($CDCl_3$) δ 1.05 (d, J = 6.5 Hz, 12H, CH_3), 1.44 (s, 9H, CH_3), 2.20 (m, 2H, CH), 2.40 (d, J = 7.1 Hz, 4H, CH_2), 2.97 and 3.15 (m, 2H, CH_2), 4.33 (m, 1H, CH), 5.14 (m, 1H, NH), 5.31 and 5.81 (br, 2H, NH_2), 7.05 (s, 1H, 6-ArH), 7.11 (s, 2H, 2-ArH and 5-ArH). ESI-MS: m/z 465 (MH^+).

*4.1.8.3. (S)-tert-butyl 1-amino-3-(3,4-bis(2,2-dimethylbutanoyloxy) phenyl)-1-oxopropan-2-ylcarbamate **(8c)**.* Yield 68%; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7.4 Hz, 6H, CH_3), 1.28 (s, 12H, CH_3), 1.44 (s, 9H, CH_3), 1.70 (q, J = 7.4 Hz, 4H, CH_2), 2.94 and 3.16 (m, 2H, CH_2), 4.32 (m, 1H, CH), 5.15 (m, 1H, NH), 5.31 and 5.74 (br, 2H, NH_2), 6.98 (s, 1H, 6-ArH), 7.07 (m, 2H, 2-ArH and 5-ArH). ESI-MS: m/z 493 (MH^+).

*4.1.8.4. (S)-tert-butyl 1-amino-3-(3,4-bis(3,3-dimethylbutanoyloxy) phenyl)-1-oxopropan-2-ylcarbamate **(8d)**.* Yield 75%; ^{1}H NMR ($CDCl_3$) δ 1.12 (s, 18H, CH_3), 1.43 (s, 9H, CH_3), 2.41 (s, 4H, CH_2), 2.98 and 3.13 (m, 2H, CH_2), 4.34 (m, 1H, CH), 5.14 (m, 1H, NH), 5.37 and 5.83 (br, 2H, NH_2), 7.04 (s, 1H, 6-ArH), 7.11 (s, 2H, 2-ArH and 5-ArH). ESI-MS: m/z 493 (MH^+).

*4.1.8.5. (S)-tert-butyl 1-amino-3-(3,4-bis(ethoxycarbonyloxy)phenyl)-1-oxopropan-2-ylcarbamate **(8e)**.* Yield 65%; ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 6H, CH_3), 1.43 (s, 9H, CH_3), 2.96 and 3.15 (m, 2H, CH_2), 4.20 (q, 4H, CH_2), 4.33 (m, 1H, CH), 5.14 (m, 1H, NH), 5.35 and 5.80 (br, 2H, NH_2), 7.04 (s, 1H, 6-ArH), 7.10 (s, 2H, 2-ArH and 5-ArH). ESI-MS: m/z 441 (MH^+).

*4.1.9. General preparation of the hydrochlorides of diacyl L-dopa amide derivatives (**9a**–**9e**)*

To a solution of **8** (1 mmol) in CH_2Cl_2 (6 mL) cooled on an ice-bath was added HCl solution (2 mL, 4 M in dioxane). The solution was stirred for 2 h. After removal of the solvent, product **9** was obtained as a white solid.

*4.1.9.1. (S)-3-(3,4-bis(pivaloyloxy)phenyl)-1-amino-1-oxopropan-2-ammonium chloride **(9a)**.* Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 1.27 and 1.28 (s, 18H, CH_3), 3.00–3.15 (m, 2H, CH_2), 3.99 (m, 1H, CH), 7.17 (s, 1H, ArH), 7.20 (s, 2H, ArH), 7.58 and 7.99 (s, 2H, NH_2), 8.30 (br, 3H, NH_3^+). ESI-MS: m/z 365 ($[M-Cl]^+$). HRMS: Calcd for $C_{19}H_{29}N_2O_5$: 305.2076 ($[M-HCl+H]^+$), found: 305.2067.

*4.1.9.2. (S)-3-(3,4-bis(3-methylbutanoyloxy)phenyl)-1-amino-1-oxopropan-2-ammonium chloride **(9b)**.* Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 0.99 (m, 12H, CH_3), 2.06 (m, 2H, CH), 2.44 (m, 4H, CH_2), 3.02 and 3.13 (m, 2H, CH_2), 3.97 (m, 1H, CH), 7.18 (s, 1H, ArH), 7.22 (s, 2H, ArH), 7.60 and 8.00 (s, 2H, NH_2), 8.26 (br, 3H, NH_3^+). ESI MS: 365 ($[M-Cl]^+$), 387 ($[M-HCl+Na]^+$). HRMS: Calcd for $C_{19}H_{29}N_2O_5$: 305.2076 ($[M-HCl+H]^+$), found: 305.2068.

*4.1.9.3. (S)-3-(3,4-bis(2,2-dimethylbutanoyloxy)phenyl)-1-amino-1-oxopropan-2-ammonium chloride **(9c)**.* Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 0.92 (m, 6H, CH_3), 1.24 and 1.25 (s, 12H, CH_3), 1.66 (m, 4H, CH_2), 3.02 and 3.14 (m, 2H, CH_2), 3.99 (t, J = 6.5 Hz, 1H, CH), 7.15–7.22 (m, 3H, ArH), 7.60 and 7.99 (s, 2H, NH_2), 8.25(br, 3H, NH_3^+). ESI MS: 393 ($[M-Cl]^+$), 410 ($[M-HCl+NH_4]^+$), 415 ($[M-HCl+Na]^+$). HRMS: Calcd for $C_{21}H_{33}N_2O_5$: 393.2389 ($[M-HCl+H]^+$), found: 393.2400.

*4.1.9.4. (S)-3-(3,4-bis(3,3-dimethylbutanoyloxy)phenyl)-1-amino-1-oxopropan-2-ammonium chloride **(9d)**.* Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 1.07 and 1.08 (s, 18H, CH_3), 2.44 and 2.45 (s, 4H, CH_2), 3.06 and 3.14 (m, 2H, CH_2), 4.00 (m, 1H, CH), 7.18 (s, 1H, ArH), 7.22 (s, 2H, ArH), 7.60 and 8.03 (br, 2H, NH_2), 8.28 (br, 3H, NH_3^+). ESI MS: 393 ($[M-Cl]^+$), 415 ($[M-HCl+Na]^+$). HRMS: Calcd for $C_{21}H_{33}N_2O_5$: 393.2389 ($[M-HCl+H]^+$), found: 393.2386.

*4.1.9.5. (S)-3-(3,4-bis(ethoxycarbonyloxy)phenyl)-1-amino-1-oxopropan-2-ammonium chloride **9e**.* Quantitative yield; ^{1}H NMR (DMSO-d_6) δ ^{1}H NMR (DMSO-d_6) δ1.29 (t, J = 3.5 Hz, 6H, CH_3), 3.06 and 3.15 (m, 2H, CH_2), 4.00 (m, 1H, CH), 4.25 (q, J = 4.0 Hz, 4H, CH_2), 7.27 (s, 1H, ArH), 7.32 (d, J = 2.0 Hz, 1H, ArH), 7.38 (d, J = 8.5 Hz, 1H, ArH), 7.60 and 8.01 (s, 2H, NH_2), 8.24 (br, 3H, NH_3^+). ESI MS: 341 ($[M-Cl]^+$), 363 ($[M-HCl+Na]^+$). Calcd for $C_{15}H_{21}N_2O_7$: 341.1348 ($[M-HCl+H]^+$), found: 341.1339.

*4.1.10. General preparation of the hydrochlorides of L-dopa amide derivatives (**10b**–**10g**)*

To a suspension of **6** (9.60 mmol) in MeOH/EtOAc (1:1, 120 mL) was added 5% Pd/C (20% weight of **6**), followed by the addition of HCl solution (12 mL, 1.25 M in MeOH). Hydrogenation was carried out under 30 psi H_2 at room temperature for 3 h. After filtration to remove the catalyst, the filtrate was concentrated. The residue was redissolved in MeOH (6 mL) and then diethyl ether was added to precipitate the product, which was collected as a white solid.).

*4.1.10.1. (S)-1-amino-3-(3,4-dihydroxyphenyl)-1-oxopropan-2-ammonium chloride **(10b)**.* Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 2.75-2.81 and 2.88-2.94 (m, 2H, CH_2), 4.15 (m, 1H, CH), 6.51 (dd, J = 8.0 and 1.9 Hz, 1H, 6-ArH), 6.66 (m, 2H, 2-ArH and 5-ArH), 7.50 and 7.90 (s, 2H, NH_2), 8.01 (br, 3H, NH_3^+), 8.90 and 8.94 (s, 2H, OH). ESI MS: 197 ($[M-Cl]^+$).

*4.1.10.2. (S)-3-(3,4-dihydroxyphenyl)-1-(methylamino)-1-oxopropan-2-ammonium chloride **(10c)**.* Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 2.59 (d, J = 4.5 Hz, 3H, CH_3), 2.73-2.89 (m, 2H, CH_2), 3.78 (m, 1H, CH), 6.45 (dd, J = 8.0 and 1.9 Hz, 1H, 6-ArH), 6.60 (d, J = 1.9 Hz, 1H, 2-ArH), 6.66 (d, J = 8.0 Hz, 1H, 5-ArH), 8.21 (br, 3H, NH_3^+), 8.43 (m, 1H, NH), 8.92 (br, 2H, OH). ESI MS: 211 ($[M-Cl]^+$). HRMS: Calcd for $C_{10}H_{15}N_2O_3$: 211.1082 ($[M-HCl+H]^+$), found: 211.1081.

4.1.10.3. (S)-3-(3,4-dihydroxyphenyl)-1-(ethylamino)-1-oxopropan-2-ammonium chloride ***(10d)***. Yield 96%; ^{1}H NMR (DMSO-d_6) δ 0.96 (t, J = 7.2 Hz, 3H, CH_3), 2.70–2.85 (m, 2H, DOPA-CH_2), 2.99–3.15 (m, 2H, CH_2), 3.67 (t, J = 7.0 Hz, 1H, DOPA-CH), 6.45 (dd, J = 8.0 and 1.9 Hz, 1H, 6-ArH), 6.60 (d, J = 1.9 Hz, 1H, 2-ArH), 6.65 (d, J = 8.0 Hz, 1H, 5-ArH), 7.56 (br, 3H, NH_3^+), 8.29 (t, J = 5.3 Hz, 1H, NH), 8.88 (br, 2H, OH). ESI MS: 225 ([M−Cl]$^+$). HRMS: Calcd for $C_{11}H_{17}N_2O_3$: 225.1239 ([M−HCl+H]$^+$), found: 225.1224.

4.1.10.4. (S)-3-(3,4-dihydroxyphenyl)-1-(isopropylamino)-1-oxopropan-2-ammonium chloride ***(10e)***. Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 0.94 (d, J = 6.6 Hz, 3H, CH_3), 1.05 (d, J = 6.6 Hz, 3H, CH_3), 2.79 (m, 2H, DOPA-CH_2), 3.70 (t, J = 7.0 Hz, 1H, DOPA-CH), 3.81 (m, 1H, CH), 6.45 (dd, J = 8.0 and 1.9 Hz, 1H, 6-ArH), 6.60 (d, J = 1.9 Hz, 1H, 2-ArH), 6.65 (d, J = 8.0 Hz, 1H, 5-ArH), 7.95 (br, 3H, NH_3^+), 8.18 (d, J = 7.6 Hz, 1H, NH), 8.87 and 8.90 (br, 2H, OH). ESI MS: 239 ([M−Cl]$^+$). HRMS: Calcd for $C_{12}H_{19}N_2O_3$: 239.1396 ([M−HCl+H]$^+$), found: 239.1400.

4.1.10.5. (S)-3-(3,4-dihydroxyphenyl)-1-(dimethylamino)-1-oxopropan-2-ammonium chloride ***(10f)***. Quantitative yield; ^{1}H NMR (DMSO-d_6) δ 2.61 (s, 3H, CH_3), 2.74 (m, 1H, DOPA-CH_2), 2.80 (s, 3H, CH_3), 2.89 (m, 1H, DOPA-CH_2), 4.39 (m, 1H, DOPA-CH), 6.43 (dd, J = 8.0 and 1.9 Hz, 1H, 6-ArH), 6.59 (d, J = 1.9 Hz, 1H, 2-ArH), 6.68 (d, J = 8.0 Hz, 1H, 5-ArH), 8.23 (br, 3H, NH_3^+), 8.96 (br, 2H, OH). ESI MS: 225 ([M−Cl]$^+$).

4.1.10.6. (S)-3-(3,4-dihydroxyphenyl)-1-(phenethylamino)-1-oxopropan-2-ammonium chloride ***(10g)***. Yield 91%; ^{1}H NMR (DMSO-d_6) δ 2.58–2.70 (m, 2H, CH_2), 2.72–2.89 (m, 2H, DOPA-CH_2), 3.18 (m, 1H, CH_2, anther H was buried by water peak), 3.79 (t, J = 6.9 Hz, 1H, DOPA-CH), 6.45 (d, J = 7.9 Hz, 1H, 6-ArH), 6.63 (s, 1H, 2-ArH), 6.67 (d, J = 8.0 Hz, 1H, 5-ArH), 7.16–7.22 (m, 3H, PhH), 7.26–7.31 (m, 2H, PhH), 8.14 (br, 3H, NH_3^+), 8.58 (m, 1H, NH), 8.93 (br, 2H, OH). ESI MS: 301 ([M−Cl]$^+$). HRMS: Calcd for $C_{17}H_{21}N_2O_3$: 301.1552 ([M−HCl+H]$^+$), found: 301.1546.

4.1.11. General preparation of the hydrochlorides of the diacetyl L*-dopa amide derivatives (**11b**–**11f**)*

To a solution of **10** (9 mmol) in acetic acid (20 mL) was bubbled HCl gas at 40 °C, followed by the dropwise addition of acetyl chloride (12 mL). The solution was allowed to cool to room temperature and was stirred overnight. Addition of diethyl ether caused to precipitation of the product **11**, which after filtration was obtained as a white solid.

4.1.11.1. (S)-1-amino-3-(3,4-diacetoxyphenyl)-1-oxopropan-2-ammonium chloride ***(11b)***. Yield 84%; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.04 and 3.14 (m, 2H, DOPA-CH_2), 3.99 (m, 1H, CH), 7.19–7.25 (m, 3H, ArH), 7.61 and 8.04 (s, 2H, NH_2), 8.30 (br, 3H, NH_3^+). ESI MS: 281 ([M−Cl]$^+$), 303 ([M−HCl+Na]$^+$). HRMS: Calcd for $C_{13}H_{17}N_2O_5$: 281.1137 ([M−HCl+H]$^+$), found: 281.1140.

4.1.11.2. (S)-3-(3,4-diacetoxyphenyl)-1-(methylamino)-1-oxopropan-2-ammonium chloride ***(11c)***. 82% yield. ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.58 (d, J = 4.3 Hz, 3H, CH_3), 3.06 (d, J = 6.9 Hz, 2H, DOPA-CH_2), 3.98 (m, 1H, DOPA-CH), 7.13–7.23 (m, 3H, ArH), 8.43 (br, 3H, NH_3^+), 8.60 (m, 1H, NH). ESI MS: 295 ([M−Cl]$^+$). HRMS: Calcd for $C_{14}H_{19}N_2O_5$: 295.1294 ([M−HCl+H]$^+$), found: 295.1272.

4.1.11.3. (S)-3-(3,4-diacetoxyphenyl)-1-(ethylamino)-1-oxopropan-2-ammonium chloride ***(11d)***. Yield 90%; ^{1}H NMR (DMSO-d_6) δ 0.93 (t, J = 7.2 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.94-3.16 (m, 4H, DOPA-CH_2 and NCH_2), 3.95 (m, 1H, DOPA-CH), 7.13-7.22 (m, 3H, ArH), 8.44 (br, 3H, NH_3^+), 8.58 (t, J = 5.4 Hz, 1H, NH). ESI MS: 309 ([M−Cl]$^+$), 331 ([M−HCl+Na]$^+$). HRMS: Calcd for $C_{15}H_{21}N_2O_5$: 309.1450 ([M−HCl+H]$^+$), found: 309.1445.

4.1.11.4. (S)-3-(3,4-diacetoxyphenyl)-1-(isopropylamino)-1-oxopropan-2-ammonium chloride ***(11e)***. Yield 75%; ^{1}H NMR (DMSO-d_6) δ 0.88 (d, J = 6.5 Hz, 3H, CH_3), 1.04 (d, J = 6.5 Hz, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.00 and 3.12 (m, 2H, DOPA-CH_2), 3.76 (m, 1H, CH), 3.95(m, 1H, DOPA-CH), 7.13-7.25 (m, 3H, ArH), 8.42 (d, J = 7.6 Hz, 1H, NH), 8.47 (br, 3H, NH_3^+). ESI MS: 323 ([M−Cl]$^+$), 345 ([M−HCl+Na]$^+$). HRMS: Calcd for $C_{16}H_{23}N_2O_5$: 323.1607 ([M−HCl+H]$^+$), found: 323.1601.

4.1.11.5. (S)-3-(3,4-diacetoxyphenyl)-1-(dimethylamino)-1-oxopropan-2-ammonium chloride ***(11f)***. Yield 92%; ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 2.94 and 3.16 (m, 2H, DOPA-CH_2), 4.54 (m, 1H, DOPA-CH), 7.11–7.23 (m, 3H, ArH), 8.50 (br, 3H, NH_3^+). ESI MS: 309 ([M−Cl]$^+$), 331 ([M−HCl+Na]$^+$). HRMS: Calcd for $C_{15}H_{21}N_2O_5$: 309.1450 ([M−HCl+H]$^+$), found: 309.1445.

4.2. Pharmacological evaluation

4.2.1. Animals

Male Wistar rats (250–500g, Harlan Ltd.). Animals were housed in groups of 4 on a 12-h light-dark cycle with an environment of 50% humidity and a temperature of 21 ± 2 °C in accordance with Animals (Scientific Procedures) Act 1996 Home Office regulations. Rats had access to food and water *ad libitum*. All animals used in this study were treated in accordance with the UK 1986 Animals (Scientific Procedures Act).

4.2.2. Pharmacological and pharmacokinetic evaluation

4.2.2.1. Surgical procedures. Male Wistar rats were treated with desipramine hydrochloride (25 mg/kg ip, 30 min prior to 6-OHDA) to prevent the destruction of noradrenergic fibres. Rats were anaesthetized in an induction chamber using isofluorane (1–2% in 95% O_2, 5% CO_2 carrier gas), placed in a Kopf stereotaxic frame and anaesthesia maintained with 0.5–1.0% isofluorane. An incision was made in the scalp and a 0.8-mm-diameter hole made in the skull at coordinates AP: −0.26mm L: +2.0 mm (all co-ordinated measured from bregma). The neurotoxin 6-hydroxydopamine hydrobromide (6-OHDA) (8 μg free base in 4 μL of 0.9% saline containing 0.05% ascorbic acid) was injected into the left median forebrain bundle at a constant rate over 4 min (1 μL/min) using a 10-μL Hamilton syringe lowered to −8 mm below the dura. The needle remained in place for a further 4 min before being removed, and the wound cleaned and sutured. Flunixin hydrochloride (2.5 mg/kg, Dunlop's Veterinary Supplies, Dumfries, UK) was administered for pain relief and a rehydration treatment of 5% glucose in 0.9% saline (up to 5 mL ip) was given prior to recovery from the anaesthetic.

4.2.2.2. Confirmation of lesion of the nigro-striatal tract. At least 2 weeks following surgery, animals were assessed for rotational behaviour (see below) in response to the administration of apomorphine hydrochloride (0.5 mg/kg s.c. in 0.9% saline containing 0.05% ascorbic acid) to evaluate the extent of the lesion. Only those rats exhibiting >6 turns/min at peak activity were used in subsequent studies.

4.2.2.3. Assessment of the induction of rotational activity by test compounds. At least 1 week after apomorphine administration, rats (n=4–8 per treatment) were assessed for rotational activity with either L-dopa or a test drug. Compounds were administered either via the intraperitoneal (ip) route or orally by gavage (po). Animals

were treated with the peripheral decarboxylase inhibitor benserazide (10 mg/kg) to prevent degradation of L-dopa to dopamine in peripheral tissues and placed in rotometers (Med Associates) for up to 30 min to measure basal rotational activity. They were then treated with L-dopa or a test compound at a single fixed molar equivalent dose (63.4 μmole/kg ip or po). Rotational behaviour was assessed for up to 4 hours after test drug/L-dopa administration. Animals were typically treated with a series of compounds for comparative purposes with each treatment being administered at least 1 day apart.

4.2.2.4. Data analysis. The number of rotations measured per 10 min over the 4-hour period was determined. Animals were considered active if they turned >10 turns per 10 min. From this data the following parameters were measured:

A: Total activity (AUC activity, where AUC = area under the locomotor activity/time curve)
B: Peak activity
C: Duration of activity

Values are quoted as % of L-dopa induced effects.

4.2.3. Pharmacokinetic analysis

4.2.3.1. Pharmacokinetics dosing protocol. Naïve male Wistar rats (250–500 g) were used for the pharmacokinetic studies. Animals were fasted overnight. Test compounds were dissolved in 0.9% saline and co-dosed with benserazide (10 mg/kg) at a molecular weight equivalent dose to 12.5 mg/kg L-dopa. Blood samples were taken via a butterfly needle located into the lateral tail vein and collected into sample tubes containing heparin as the anti-coagulant. Blood samples were centrifuged at 5000 rpm for 10 min; the supernatant plasma was removed and stored at −80 °C.

4.2.3.2. Preparation of sample and standard solutions. Stock solutions of 10 mM L-dopa and warfarin were prepared in 20% TFA, 10 mM sodium meta bisulphite and DMSO, respectively.

Standard curves and Quality control (QC) samples were prepared by spiking control rat plasma with L-dopa to achieve an initial concentration of 50 μM. Serial dilutions of this solution were performed in rat plasma to result in solutions with 25, 6.25, 3.125, 1.56, 0.78 and 0.39 μM L-dopa.

One volume of sample plasma, standard and QC sample plasma was transferred from each sample vial to a 96 well plate. Compounds were extracted from plasma by addition of one volume of 20% TFA in 10 mM sodium metabisulphite containing the internal standard, warfarin, at 0.5 μM. The samples were vortex mixed and centrifuged at 4500 rpm for 4 min to precipitate plasma proteins. One volume of water was added to each well and the protein pellet was re-suspended. The samples were again vortex mixed and centrifuged at 4500 rpm for 9 min to precipitate plasma proteins.

The supernatant was analysed by LC-MS/MS method which is described in detail below.

4.2.3.3. LC-MS/MS analysis. The LC-MS/MS system consisted of an Agilent 1100 series gradient HPLC pump (Agilent Technologies, Palo Alto, CA), a CTC HTS PAL Autosampler (CTC Analytics, Zwingen, Switzerland) and an Applied Biosystems/MDS Sciex API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a turbo ionspray interface and operated in positive electrospray mode. Analytes in incubation mixtures were separated by reverse phase HPLC using a Phenomenex Sphereclone ODS 2 column (150 × 4.6 mm, 3 μm, Phenomenex, Torrance, CA).

A gradient elution program was used at a flow rate of 1 ml/min with a mobile phase consisting of acetonitrile/0.1% formic acid (5% v/v) in water/0.1% formic acid, delivered for 1.5 min, after which time the acetonitrile concentration was increased to 95% over 0.5 min and held at 95% for four minutes before restoring it back to 5% for the remaining two minutes. The injection volume was 20 μl. Approximately 10% of the eluent was introduced into the mass spectrometer source. The source temperature of the mass spectrometer was maintained at 450 °C, and other source parameters (e.g., collision energy, declustering potential, curtain gas pressure etc.) were optimised on the day of analysis to achieve maximum sensitivity. Quantification of L-dopa and warfarin was achieved by monitoring the transitions of $m/z = 198.075/152.1$ and $m/z = 369.069/163$, respectively.

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