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Research Paper

Novel L-Dopa and Dopamine Prodrugs Containing a 2-Phenyl-imidazopyridine Moiety

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Purpose. The aim of this study was to gain insight into the feasibility of enhancing the delivery of L-Dopa and dopamine to the brain by linking these neurotransmitters and L-Dopa ethyl ester to 2-phenyl-3-carboxymethyl-imidazopyridine compounds giving rise to the so-called Dopimid compounds.
Materials and Methods. A number of Dopimid compounds were synthesized and both stability and binding studies to dopaminergic and benzodiazepine receptors were performed. To evaluate whether Dopimid compounds are P-gp substrates, [^{3}H]ritonavir uptake experiments and bi-directional transport studies on confluent MDCKII-MDR1 monolayers were carried out. The brain penetration properties of Dopimid compounds were estimated by the Clark's computational model and evaluated by investigation of their transport across BBMECs monolayers. The dopamine levels following the intraperitoneal administration of the selected Dopimid compounds were measured in vivo by using brain microdialysis in rat.
Results. Tested compounds were adequately stable in solution buffered at pH 7.4 but undergo faster cleavage in dilute rat serum at 37°C. Receptor binding studies showed that Dopimid compounds are essentially devoid of affinity for dopaminergic and benzodiazepine receptors. [^{3}H]ritonavir uptake experiments indicated that selected Dopimid compounds, like L-Dopa and dopamine hydrochloride, are not substrates of P-gp and it was also confirmed by bi-directional transport experiments across MDCKII-MDR1 monolayers. By Clark's model a significant brain penetration was deduced for L-Dopa ethyl ester and dopamine derivatives. Transport studies involving BBMECs monolayers indicated that some of these compounds should be able to cross the BBB. Interestingly, the rank order of apparent permeability (P_{app}) values observed in these assays parallels that calculated by the computational approach. Brain microdialysis experiments in rat showed that intraperitoneal acute administration of some Dopimid compounds induced a dose-dependent increase in cortical dopamine output.
Conclusion. Based on these results, it may be concluded that some Dopimid compounds can be proposed as novel L-Dopa and dopamine prodrugs.

KEY WORDS: blood brain barrier; imidazopyridine-compounds; L-Dopa and dopamine; microdialysis; prodrugs.

INTRODUCTION

L-Dopa (3,4-dihydroxyphenyl-L-alanine) is a precursor of dopamine (DA), which is deficient in the brains of patients suffering from the progressive disorder of the central nervous system (CNS) known as Parkinson's disease (PD). Peripheral administration of DA cannot be useful for the treatment of PD since DA is unable to cross the blood brain barrier (BBB) due to its hydrophilic nature and the absence of active transport mechanism (1). Instead, L-Dopa enters into the CNS through active transport and it is enzymatically decarboxylated in the brain giving rise to DA. Actually, L-Dopa can be considered a prodrug of DA. However, L-Dopa is characterized by a low water solubility, sensitive to chemical and enzymatic oxidations and suffers from extensive peripheral decarboxylation (2,3). After a good initial response, complications are associated with long-term L-Dopa therapy including motor fluctuations, dyskinesias, mental changes and loss of efficacy (4). In this regard, it has been suggested that drugs enhancing the γ-aminobutyric acid (GABA) transmission could be helpful in PD (5). In fact, it has been suggested that $GABA_A$ receptors contribute to modulation of the activity of mesocortical and mesolimbic dopaminergic neurons (6).

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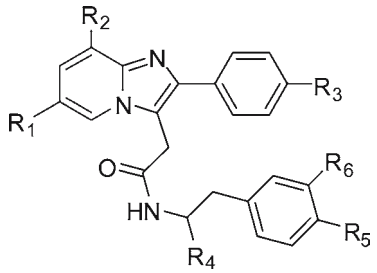
Among the strategies aimed at enhancing the delivery of biologically active substances to the brain, the prodrug approach is commonly used to improve physicochemical, biopharmaceutical, and drug delivery properties. Thus, a promoiety is covalently attached to the bioactive molecule, and the resulting prodrug is converted to the parent drug in the body exhibiting the pharmacological effect. Therefore, it may be useful to manipulate the molecules L-Dopa and DA to obtain compounds with increased lipophilicity, and thereby allow them to gain access to the CNS. In this paper, we describe the synthesis and properties of compounds **1–8** and **9–12** (Dopimid compounds, Table I) which are characterized by an L-Dopa or DA moiety, respectively, and linked to appropriately substituted 2-phenyl-imidazopyridine-3-acetic acids. These potential prodrugs have been prepared in order to take advantage of two features concerning imidazopyridine compounds. The first one is of a pharmacodynamic nature and is based on the high affinity and selectivity for the GABA-benzodiazepine receptor (GABA-BZR) complex shown by most phenyl-imidazopyridine compounds, as exemplified by alpidem and zolpidem (7,8). The second feature is that the lipophilic phenyl-imidazopyridine moiety could serve as a carrier for L-Dopa and DA leading to increased brain levels of these last two compounds taking into account of the high blood brain barrier (BBB) crossing ability shown by most phenyl-imidazopyridine derivatives (e.g., log C_{brain}/C_{blood} of alpidem is 0.4, (8)). Moreover, it has been reported that selective gabaergic agonists such as zolpidem could be useful in patients who have complications associated with long-term L-Dopa treatment (9). Therefore, we considered to combine by covalent-bonding of L-Dopa and DA to 2-phenyl-imidazopyridine-3-acetic acids. The rationale was to have pharmacologically inert prodrugs, which upon delivery into the brain, are cleaved to the active L-Dopa and DA and phenyl-imidazopyridine moieties. These last could interact at GABA-BZR complex and it can influence the dopaminergic neurons. A number of advantages were expected as good lipophilicity, BBB penetration, delivery enhancement at the target site and reduced complications associated with L-Dopa treatment. Synthesis, *in vitro* studies for assessing the BBB penetration, binding data and results of a biodistribution study are herein reported and discussed.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. IR spectra were

Table I. Chemical Structures of Compounds **1**–**12**, **16 a–d** and **19 a–d**



Dopimid Compound	R_1	R_2	R_3	R_4	R_5	R_6	Mp (°C)	Yield (%)
1	Cl	Cl	Cl	COOH	OH	OH	168–173	95
2	Cl	H	Cl	COOH	OH	OH	212 dec.	96
3	Cl	Cl	H	COOH	OH	OH	230 dec.	98
4	Cl	H	H	COOH	OH	OH	165–170	96
5	Cl	Cl	Cl	$COOCH_2CH_3$	OH	OH	107–110	63[a]
6	Cl	H	Cl	$COOCH_2CH_3$	OH	OH	210 dec.	85[a]
7	Cl	Cl	H	$COOCH_2CH_3$	OH	OH	87–90	73[a]
8	Cl	H	H	$COOCH_2CH_3$	OH	OH	168–170	77[a]
9	Cl	Cl	Cl	H	OH	OH	147–150	32
10	Cl	H	Cl	H	OH	OH	127–131	45
11	Cl	Cl	H	H	OH	OH	125–130	35
12	Cl	H	H	H	OH	OH	85–90	40
16a	Cl	Cl	Cl	$COOCH_2CH_3$	OTBDMS	OTBDMS	87–90	45
16b	Cl	H	Cl	$COOCH_2CH_3$	OTBDMS	OTBDMS	90–93	30
16c	Cl	Cl	H	$COOCH_2CH_3$	OTBDMS	OTBDMS	110–115	21
16d	Cl	H	H	$COOCH_2CH_3$	OTBDMS	OTBDMS	105–107	44
19a	Cl	Cl	Cl	H	OTBDMS	OTBDMS	183–185	27
19b	Cl	H	Cl	H	OTBDMS	OTBDMS	165–167	40
19c	Cl	Cl	H	H	OTBDMS	OTBDMS	147–150	34
19d	Cl	H	H	H	OTBDMS	OTBDMS	156–161	35

[a] Yields obtained following Method B.

obtained on a Perkin–Elmer 283 spectrophotometer (KBr pellets for solids or nujol for liquids). ^{1}H NMR spectra were determined on a Varian VX Mercury operating at 300 MHz instrument. Chemical shifts are given in δ values downfield from Me_4Si as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c GC-MS low-resolution spectrometer or were obtained using an Agilent 1100 LC-MSD trap system VL instrument using methanol/7 mM ammonium formiate 9/1 (v/v). Elemental analyses were carried out with a Hewlett Packard 185 C, H, N analyzer and results were within ±0.40% of the theoretical values. Silica gel 60 (Merck 70–230 mesh) was used for column chromatography. All the following reactions were performed under a nitrogen atmosphere and the progress of the reaction was monitored by thin-layer chromatography (TLC) by using Kieselgel 60 F254 (Merck) plates.

The preparation of the starting 2-phenylimidazo[1,2-a]pyridine-3-acetic acids **15 a–d** was accomplished following a reported procedure (10). Dopamine hydrochloride **17**, L-Dopa, tert-butyldimethylsilyl chloride (TBDMSCl), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), ethyl-1,2-dihydro-2-ethoxy-1-quinolinecarboxylate (EEDQ), 1,1′-carbonyldiimidazole (CDI), triethylamine (TEA) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (Italy).

Reagents used for the preparation of the buffers were of analytical grade. Fresh deionized water from all glass apparatus was used in the preparation of all the solutions.

Synthesis of Ethyl 2-amino-3-[3,4-bis (tert-butyldimethylsilyloxy)phenyl]propanoate 14

The preparation of the compound **14** (Fig. 1) was accomplished according to a previously reported method (11). Briefly, a stirred mixture of L-Dopa (1 g, 5.07 mmol) in ethanol (40 ml) was saturated with HCl gas and heated at 50°C for 3 h. Excess of HCl gas was removed under a stream of nitrogen and then the solvent was evaporated under reduced pressure. The resulting residue was dried under vacuum to provide compound **13** in quantitative yield (12). To a stirred suspension of L-Dopa ethyl ester hydrochloride **13** (2.3 g, 8.8 mmol) in acetonitrile (20 ml), TEA (1.2 ml, 8.8 mmol) was added. After cooling to 0°C, TBDMSCl (4 g, 26 mmol) and DBU (3.9 ml, 26 mmol) were added. Stirring was continued for additional 18 h at room temperature (RT) and the progress of the reaction was monitored by TLC [chloroform/ethyl acetate 7/1 (v/v) as eluent]. The solvent was removed under reduced pressure and then chloroform was added to the residue. The organic phase was washed with water, dried (Na_2SO_4) and evaporated to dryness. The crude ester **14** was purified on silica gel column chromatography [chloroform/ethyl acetate 7/1 (v/v) as eluent].

General Procedure for Preparation of Dopimid Compounds 5–8

Method A (Fig. 1). To a stirred solution of the required imidazo[1,2]pyridine-3-acetic acid **15 a–d** (1.4 mmol) in anhydrous THF (20 ml), EEDQ (0.41 g, 1.68 mmol) and coumpound **14** (0.63 g, 1.4 mmol) were added. After 10 min, TEA (0.3 ml, 2.1 mmol) was added drop by drop, and stirred for prolonged time at RT for 15 h. The progress of reaction was monitored by TLC [petroleum ether/ethyl acetate 7/3 (v/v) as eluent]. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate 7/3 (v/v) as eluent. In this way, the corresponding 3,4-bis(tert-butyldimethylsilyloxy)-L-Dopa ethyl ester compounds **16 a–d** were obtained. Subsequently, complete deprotection of TBDMS groups was accomplished by using TFA. In short, the appropriate compound **16 a–d** (0.46 mmol) was dissolved in 5 ml of 95% aqueous TFA. The reaction was carried out under nitrogen atmosphere for 12–24 h at RT and the progress of the reaction was monitored by TLC [petroleum ether/ethyl acetate 7/3 (v/v) as eluent]. Then, the mixture was evaporated at RT under a stream of nitrogen and the crude product was taken up with diethyl ether. The organic phase was evaporated under reduced pressure to give the corresponding L-Dopa ethyl ester compounds **5–8.**

Method B (Fig. 1). A solution of the suitably substituted imidazo[1,2-a]pyridine-3-acetic acid **15 a–d** (1.4 mmol) and CDI (0.34 g, 2.1 mmol) in anhydrous DMF (20 ml) was stirred at RT. After 15 min, L-Dopa ethyl ester hydrochloride **13** (0.55 g, 2.1 mmol) was added, and the stirring was prolonged for 8 h. Solvent was evaporated under reduced pressure and the residue purified on silica gel column chromatography [ethyl acetate/petroleum ether 8/2 (v/v) as eluent] to give the appropriate L-Dopa ethyl ester conjugate **5–8**.

Ethyl-2-{2-[6,8-dichloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-yl]acetylamino}-3-[3,4-bis(tert-butyldimethylsilyloxy)phenyl]propanoate (16a). IR (KBr): 1,738, 1,678 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.14 (s, 6 H, CH_3Si), 0.16 (s, 6 H, CH_3Si), 0.95 (s, 9 H, $C(CH_3)_3$), 0.97 (s, 9 H, $C(CH_3)_3$), 1.29 (m, 3 H, CH_3CH_2), 2.9–3.1 (m, 2 H, CH_2CH), 3.98 (s, 2 H, CH_2CONH), 4.24 (m, 2 H, CH_2CH_3), 4.78 (m, 1 H, $CHCH_2$), 6.02 (br d, 1 H, NHCO), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.3–7.6 (m, 5 H, Ar), 8.08 (d, 1 H, Ar).

Ethyl-2-{2-[6-chloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-yl]acetylamino}-3-[3,4-bis(tert-butyldimethylsilyloxy)phenyl]propanoate (16b). IR (KBr): 3,289, 1,737, 1,650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.12 (s, 6 H, CH_3Si), 0.13 (s, 6 H, CH_3Si), 0.94 (s, 9 H, $C(CH_3)_3$), 0.96 (s, 9 H, $C(CH_3)_3$), 1.22 (t, 3 H, CH_3CH_2), 2.9–3.1 (m, 2 H, CH_2CH), 3.85 (s, 2 H, CH_2CONH), 4.1–4.2 (m, 2 H, CH_2CH_3), 4.78 (m, 1 H, $CHCH_2$), 6.09 (br d, 1 H, NHCO), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.2–7.5 (m, 6 H, Ar), 8.06 (d, 1 H, Ar).

Ethyl-2-{2-[6,8-dichloro-2-phenylimidazo[1,2-a]pyridine-3-yl]acetylamino}-3-[3,4-bis(tert-butyldimethylsilyloxy)phenyl]propanoate (16c). IR (KBr): 3,325, 1,738, 1,674 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.13 (s, 6 H, CH_3Si), 0.14 (s, 6 H, CH_3Si), 0.95 (s, 9 H, $C(CH_3)_3$), 0.97 (s, 9 H, $C(CH_3)_3$), 1.22 (t, 3 H, CH_3CH_2), 2.9–3.0 (m, 2 H, CH_2CH), 3.88 (s, 2 H, CH_2CONH), 4.1–4.2 (m, 2 H, CH_2CH_3), 4.7–4.8 (m, 1 H, $CHCH_2$), 6.01 (br d, 1 H, NHCO), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.3–7.6 (m, 6 H, Ar), 8.09 (d, 1 H, Ar).

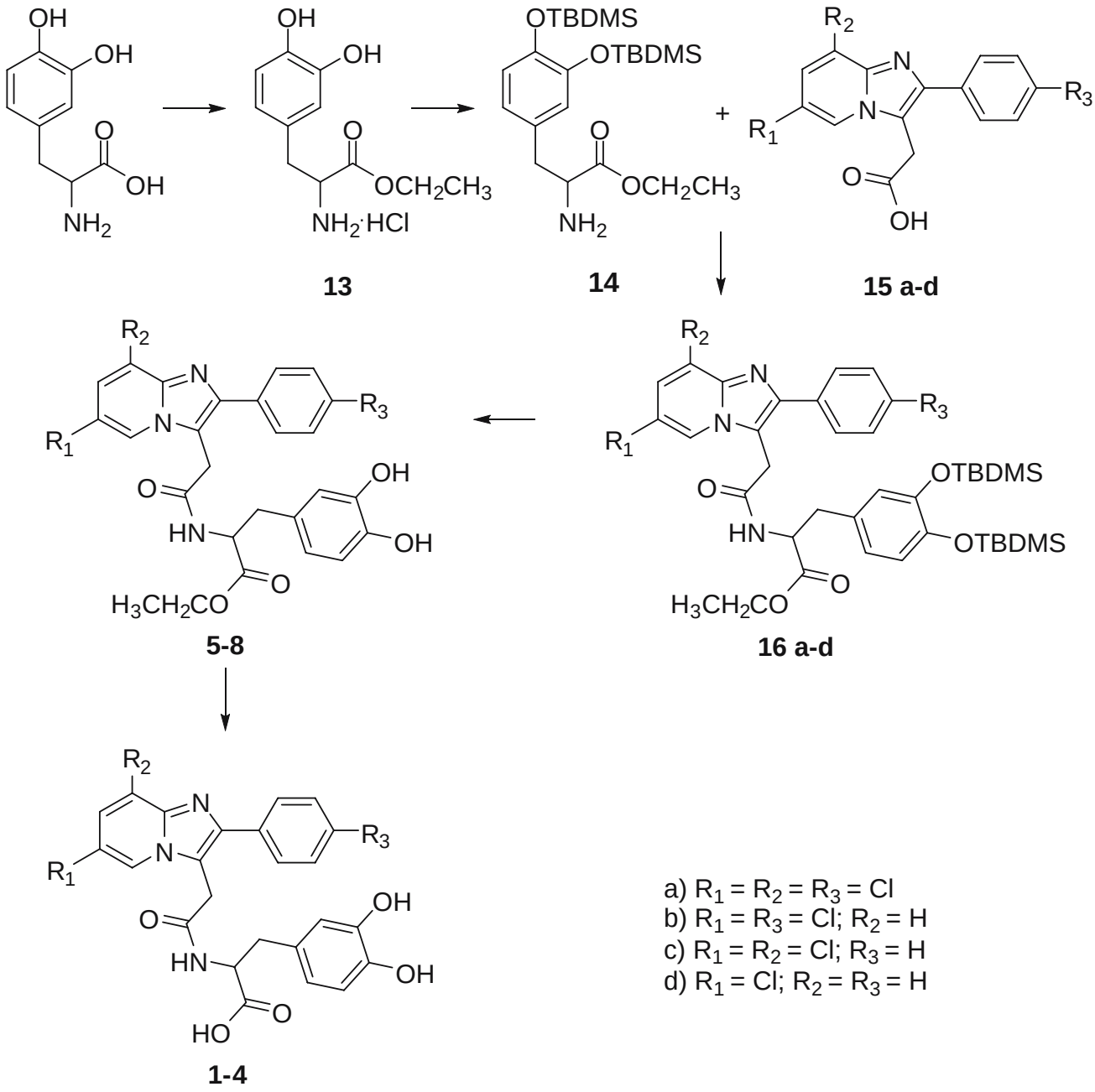
Ethyl-2-{2-[6-chloro-2-phenylimidazo[1,2-a]pyridine-3-yl]acetylamino}-3-[3,4-bis(tert-butyldimethylsilyloxy)phenyl]-propanoate (16d). IR (KBr): 3,314, 1,740, 1,682 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.13 (s, 6 H, CH_3Si), 0.15 (s, 6 H, CH_3Si), 0.94 (s, 9 H, $C(CH_3)_3$), 0.96 (s, 9 H, $C(CH_3)_3$), 1.20 (t, 3 H, CH_3CH_2), 2.8–3.0 (m, 2 H, CH_2CH), 3.90 (s, 2 H, CH_2CONH), 4.16 (q, 2 H, CH_2CH_3), 4.7–4.9 (m, 1 H, $CHCH_2$), 6.05 (br d, 1 H,

NHCO), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.2–7.7 (m, 7 H, Ar), 8.09 (d, 1 H, Ar).

Ethyl-2-{2-[6,8-dichloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoate (5). IR (KBr): 3,324, 1,732, 1,660 cm^{-1}; ^1H NMR (DMSO d_6) δ: 1.2–1.3 (m, 3 H, CH_3CH_2), 2.7–2.9 (m, 2 H, CH_2CH), 3.9–4.2 (m, 4 H, CH_2), 4.65 (m, 1 H, $CHCH_2$), 5.97 (br d, 1 H, NHCO), 6.0–6.6 (m, 3 H, Ar, L-Dopa moiety), 7.3–7.6 (m, 5 H, Ar), 8.08 (d, 1 H, Ar); MS (ESI) *m/z* 562 $[M+H]^+$.

Ethyl-2-{2-[6-chloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoate (6). IR (KBr): 3,318, 1,732, 1,660 cm^{-1}; ^1H NMR

Method A



a) $R_1 = R_2 = R_3 = Cl$
b) $R_1 = R_3 = Cl$; $R_2 = H$
c) $R_1 = R_2 = Cl$; $R_3 = H$
d) $R_1 = Cl$; $R_2 = R_3 = H$

Method B

13 + 15 a-d ⟶ **5-8**

Fig. 1. Synthetic scheme for preparation of Dopimid compounds **5**–**8**.

(DMSO d_6) δ: 1.04 (t, 3 H, CH_3CH_2), 2.7–3.0 (m, 2 H, CH_2CH), 4.0–4.1 (m, 4 H, CH_2), 4.4–4.5 (m, 1 H, $CHCH_2$), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.2–7.8 (m, 6 H, Ar), 8.5–8.6 (m, 1 H, Ar), 8.81 (br d, 1 H, NHCO); MS (ESI) *m/z* 526 $[M+H]^+$.

Ethyl-2-{2-[6,8-dichloro-2-phenylimidazo[1,2-a]pyridine-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoate (7). IR (KBr): 3,401, 1,732, 1,656 cm^{-1}; ^1H NMR (DMSO d_6) δ: 1.22 (t, 3 H, CH_3CH_2), 2.9–3.0 (m, 2 H, CH_2CH), 3.8–4.0 (m, 2 H, CH_2CONH), 4.1–4.2 (m, 2 H, CH_2CH_3), 4.7–4.8 (m, 1 H, $CHCH_2$), 6.2–6.6 (m, 3 H, Ar, L-Dopa moiety), 7.3–7.6 (m, 6 H, Ar), 8.0–8.1 (m, 1 H, Ar); MS (ESI) *m/z* 526 $[M+H]^+$.

Ethyl-2-{2-[6-chloro-2-phenylimidazo[1,2-a]pyridine-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoate (8). IR (KBr): 3,335, 1,729, 1,651 cm^{-1}; ^1H NMR (DMSO d_6) δ: 1.0–1.2 (m, 3 H, CH_3CH_2), 2.8–3.0 (m, 2 H, CH_2CH), 3.9–4.1 (m, 4 H, CH_2), 4.3–4.4 (m, 1 H, $CHCH_2$), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.2–7.7 (m, 7 H, Ar), 8.4–8.5 (m, 1 H, Ar); MS (ESI) *m/z* 494 $[M+H]^+$.

General Procedure for Preparation of Dopimid Compounds 1–4

To a solution of the appropriate ethyl ester **5–8** (0.57 mmol) in dioxane (20 ml), HCl 1*N* (5 ml) was added. The mixture was stirred at 50°C for 24 h and then, the solvent was evaporated under reduced pressure. The resulting residue was taken up with aqueous $NaHCO_3$ and extracted with ethyl acetate (3×20 ml). The cooled aqueous solution was acidified with dilute HCl and the resulting precipitate was pure L-Dopa conjugate **1–4**, which was isolated by filtration.

2-{2-[6,8-dichloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoic acid (1). IR (KBr): 3,342, 1,731, 1,660 cm^{-1}; ^1H NMR (DMSO d_6) δ: 2.7–2.9 (m, 2 H, CH_2CH), 4.03 (s, 2 H, CH_2CONH), 4.37 (m, 1 H, $CHCH_2$), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.4–7.8 (m, 5 H, Ar), 8.6–8.7 (m, 1 H, Ar), 8.75 (br d, 1 H, NHCO); MS (ESI) *m/z* 534 $[M+H]^+$.

2-{2-[6-chloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoic acid (2). IR (KBr): 3,238, 1,730, 1,666 cm^{-1}; ^1H NMR (DMSO d_6) δ: 2.7–3.0 (m, 2 H, CH_2CH), 4.09 (m, 2 H, CH_2CONH), 4.3–4.4 (m, 1 H, $CHCH_2$), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.6–7.9 (m, 6 H, Ar), 8.81 (br d, 1 H, NHCO), 8.9–9.0 (m, 1 H, Ar); MS (ESI) *m/z* 500 $[M+H]^+$.

2-{2-[6,8-dichloro-2-phenylimidazo[1,2-a]pyridin-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoic acid (3). IR (KBr): 3,238, 1,730, 1,666 cm^{-1}; ^1H NMR (DMSO d_6) δ: 2.7–3.0 (m, 2 H, CH_2CH), 3.9–4.0 (m, 2 H, CH_2CONH), 4.1–4.2 (m, 1 H, $CHCH_2$), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.3–7.8 (m, 6 H, Ar), 8.0–8.2 (br m, 1 H, NHCO), 8.4–8.5 (m, 1 H, Ar); MS (ESI) *m/z* 500 $[M+H]^+$.

2-{2-[6-chloro-2-phenylimidazo[1,2-a]pyridin-3-yl]acetylamino}-3-(3,4-dihydroxyphenyl)propanoic acid (4). IR (KBr): 3,391, 1,725, 1,661 cm^{-1}; ^1H NMR (DMSO d_6) δ: 2.7–3.0 (m, 2 H, CH_2CH), 4.13 (s, 2 H, CH_2CONH), 4.3–4.5 (m, 1 H, $CHCH_2$), 6.4–6.7 (m, 3 H, Ar, L-Dopa moiety), 7.5–8.0 (m, 7 H, Ar), 8.80 (br d, 1 H, NHCO), 9.0–9.1 (m, 1 H, Ar); MS (ESI) *m/z* 466 $[M+H]^+$.

Synthesis of 2-(3,4-di-tert-butyldimethylsilyloxyphenyl) ethanamine (18)

Dopamine hydrochloride **17** (2 g, 0.0105 mol) was added to a stirred solution of TBDMSCl (4.8 g, 0.0316 mol) in anhydrous acetonitrile (20 ml). The resulting suspension under stirring was cooled with an ice bath and then DBU (4.7 ml, 0.0316 mol) was added drop-wise during 10 min reaction. The ice bath was removed after 1 h and stirred for further 18 h at RT. After this period, solvent was removed under reduced pressure to give compound **18** (Fig. 2) as an oil (3.6 g, 90% yield).

IR (Nujol): 3,404 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.15 (s, 12 H, $SiCH_3$), 0.95 (s, 18 H, $C(CH_3)_3$), 2.91 (m, 2 H, CH_2Ar), 3.11 (m, 2 H, CH_2NH_2), 6.6–6.7 (m, 3 H, Ar); MS *m/z* : 381 (M^+, 12), 193 (base).

General Procedure for Preparation of 2-(2-phenylimidazo [1,2-a]pyridine-3-yl)-N-[2-(3,4-di-tert-butyldimethylsilyloxyphenyl) ethyl]acetamide (19 a–d)

To a stirred solution of the suitably substituted 2-phenylimidazo[1,2-a]pyridine-3-acetic acid **15 a–d** (2.1 mmol) in anhydrous THF (20 ml), EEDQ (0.62 g, 2.52 mmol) and 3,4-Bis(tert-butyldimethylsilyloxy)-phenetylamine **18** (0.8 g, 2.1 mmol) were added. The reaction mixture was stirred at RT for 10 min and then TEA (0.44 ml, 3.15 mmol) was added drop-wise. Stirring was continued for additional 24 h at RT and then the solvent was evaporated under reduced pressure. The resulting crude residue was purified by column chromatography on silica gel [petroleum ether/ethyl acetate 9/1 (v/v) as eluent] to give the appropriate compound **19 a–d** (Fig. 2).

2-[6,8-dichloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-yl]-N-[2-(3,4-di-tert-butyldimethylsilyloxyphenyl)ethyl]acetamide (19a). IR (KBr): 3,306, 1,649 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.14 (s, 6 H, CH_3Si), 0.17 (s, 6 H, CH_3Si), 0.95 (s, 9 H, $C(CH_3)_3$), 0.97 (s, 9 H, $C(CH_3)_3$), 2.65 (t, $J = 6.7$ Hz, 2 H, CH_2Ar), 3.45 (q, $J = 6.7$ Hz, 2 H, CH_2NHCO), 3.83 (s, 2 H, CH_2CONH), 5.85 (br s, 1 H, NHCO), 6.4–6.7 (m, 3 H, Ar, dopamine moiety), 7.3–7.6 (m, 5 H, Ar), 8.08 (d, 1 H, Ar); Anal. ($C_{35}H_{46}O_3N_3Cl_3Si_2$) C, H, N.

2-[6-chloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-yl]-N-[2-(3,4-di-tert-butyldimethylsilyloxyphenyl)ethyl]acetamide (19b). IR (KBr): 3,293, 1,651 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.12 (s, 6 H, CH_3Si), 0.16 (s, 6 H, CH_3Si), 0.93 (s, 9 H, $C(CH_3)_3$), 0.96 (s, 9 H, $C(CH_3)_3$), 2.69 (t, 2 H, CH_2Ar), 3.49 (q, 2 H, CH_2NHCO), 3.84 (s, 2 H, CH_2CONH), 6.02 (br s, 1 H, NHCO), 6.4–6.7 (m, 3 H, Ar, dopamine moiety), 7.2–7.5 (m, 6 H, Ar), 8.06 (m, 1 H, Ar); Anal. ($C_{35}H_{47}O_3N_3Cl_2Si_2$) C, H, N.

2-[6,8-dichloro-2-phenylimidazo[1,2-a]pyridine-3-yl]-N-[2-(3,4-di-tert-butyldimethylsilyloxyphenyl)ethyl]acetamide (19c). IR (KBr): 3,304, 1,650 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.14 (s, 6 H, CH_3Si), 0.16 (s, 6 H, CH_3Si), 0.95 (s, 9 H, $C(CH_3)_3$), 0.98 (s, 9 H, $C(CH_3)_3$), 2.64 (t, 2 H, CH_2Ar), 3.46 (q, 2 H, CH_2NHCO), 3.86 (s, 2 H, CH_2CONH), 5.85 (br s, 1 H, NHCO), 6.4–6.7 (m, 3 H, Ar, dopamine moiety), 7.3–7.6 (m, 6 H, Ar), 8.09 (m, 1 H, Ar); Anal. ($C_{35}H_{47}O_3N_3Cl_2Si_2$) C, H, N.

2-[6-chloro-2-phenylimidazo[1,2-a]pyridine-3-yl]-N-[2-(3,4-di-tert-butyldimethylsilyloxyphenyl)ethyl]acetamide (19d). IR (KBr): 3,295, 1,655 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.13 (s, 6 H, CH_3Si), 0.16 (s, 6 H, CH_3Si), 0.94 (s, 9 H, $C(CH_3)_3$), 0.97

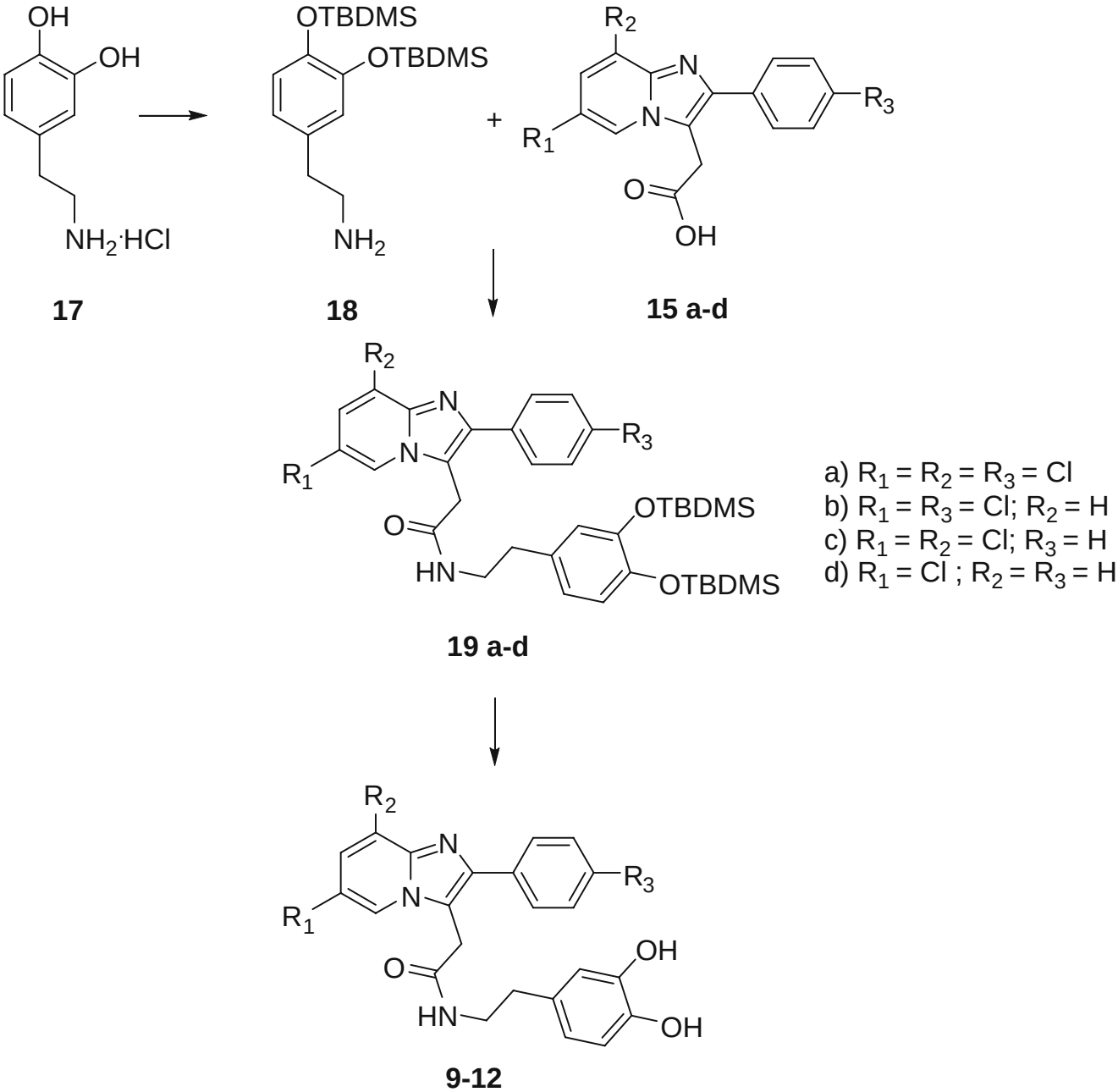


Fig. 2. Synthetic scheme for preparation of Dopimid compounds **9**–**12**.

(s, 9 H, $C(CH_3)_3$), 2.63 (t, 2 H, CH_2Ar), 3.46 (q, 2 H, CH_2NHCO), 3.90 (s, 2 H, CH_2CONH), 5.85 (br s, 1 H, NHCO), 6.4–6.7 (m, 3 H, Ar, dopamine moiety), 7.2–7.7 (m, 7 H, Ar), 8.09 (m, 1 H, Ar); Anal. ($C_{35}H_{48}O_3N_3ClSi_2$) C, H, N.

General Procedure for Preparation of 2-(2-phenylimidazo [1,2-a]pyridine-3-yl)-N-[2-(3,4-dihydroxyphenyl) ethyl]acetamide (9–12)

Complete deprotection of the 2-(2-phenylimidazo[1,2-a]pyridine-3-yl)-*N*-[2-(3,4-di-tert-butyldimethylsilyloxyphenyl)ethyl] acetamide **19 a–d** was accomplished by dissolving the suitably substituted trimethylsilyl derivative (200 mg) in 95% aqueous TFA (1.5 ml). The reaction was carried out under nitrogen atmosphere for 24 h at RT. Then, the solvent was evaporated at RT under a stream of nitrogen and the resulting residue was taken up with diethyl ether. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to give the corresponding dopamine conjugate **9–12** (Fig. 2). Yields of these Dopimid compounds were approximately quantitative.

2-[6,8-dichloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-y]-N-[2-(3,4-dihydroxyphenyl)ethyl]acetamide (9). IR (KBr) 3,295, 1,667 cm^{-1}; ^1H NMR ($DMSOd_6$) δ: 2.53 (t, 2 H, CH_2Ar), 3.24 (q, 2 H, CH_2NHCO), 3.99 (s, 2 H, CH_2CONH), 6.4–6.6 (m, 3 H, Ar, dopamine moiety), 7.5–7.9 (m, 4 H, Ar) 7.69 (d, 1 H, Ar), 8.68 (d, 1 H, Ar); MS (ESI): *m/z* 490 $[M+H]^+$; Anal. ($C_{23}H_{18}O_3N_3Cl_3$) C, H, N.

2-[6-chloro-2-(4-chlorophenyl)imidazo[1,2-a]pyridine-3-yl]-N-[2-(3,4-dihydroxyphenyl)ethyl]acetamide (10). IR (KBr) 3,305, 1,667 cm^{-1}; ^1H NMR ($DMSOd_6$) δ: 2.60 (t, 2 H, CH_2Ar), 3.33 (q, 2 H, CH_2NHCO), 4.10 (s, 2 H, CH_2CONH), 6.5–6.7 (m, 3 H, Ar, dopamine moiety), 7.6–8.0 (m, 6 H, Ar), 9.03 (m, 1 H, Ar); MS (ESI): *m/z* 456 $[M+H]^+$; Anal. ($C_{23}H_{19}O_3N_3Cl_2$) C, H, N.

2-[6,8-dichloro-2-phenylimidazo[1,2-a]pyridine-3-yl]-N-[2-(3,4-dihydroxyphenyl)ethyl]acetamide (11). IR (KBr) 3,323, 1,661 cm^{-1}; ^1H NMR ($DMSOd_6$) δ: 2.50 (t, 2 H, CH_2Ar), 3.33 (q, 2 H, CH_2NHCO), 4.10 (s, 2 H, CH_2CONH), 6.4–6.6 (m, 3 H, Ar, dopamine moiety), 7.4–7.7 (m, 6 H, Ar), 8.68 (m, 1 H, Ar); MS (ESI): *m/z* 456 $[M+H]^+$; Anal. ($C_{23}H_{19}O_3N_3Cl_2$) C, H, N.

2-[6-chloro-2-phenylimidazo[1,2-a]pyridine-3-y]-N-[2-(3,4-dihydroxyphenyl)ethyl]acetamide (12). IR (KBr) 3,293, 1,669 cm^{-1}; ^1H NMR ($DMSOd_6$) δ: 2.5–2.6 (m, 2 H, CH_2Ar), 3.2–3.3 (m, 2 H, CH_2NHCO), 4.03 (s, 2 H, CH_2CONH), 6.4–6.6 (m, 3 H, Ar, dopamine moiety), 7.5–7.9 (m, 7 H, Ar), 8.9–9.0 (m, 1 H, Ar); MS (ESI): *m/z* 422 $[M+H]^+$; Anal. ($C_{23}H_{20}O_3N_3Cl$) C, H, N.

Stability Studies

Chemical Hydrolysis

Stability studies were carried out at controlled temperature (37 ± 0.2°C) in a water bath shaken at 150 rpm. The hydrolysis of Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** was studied at pH 7.4 in 0.05 M phosphate buffer at 37°C. The experiments were carried out by adding 50 μl of a stock solution in DMSO (1.5, 2.6, 3.6, 2.8, 3.7, and 2.7 mg/ml for **1**, **2**, **5**, **6**, **9**, and **10**, respectively), to 5 ml of the buffer solution preheated at 37°C. The final concentration of the resulting solutions was about 50 μM. The test solutions of the compounds were vortexed and maintained in a shaker water bath at constant temperature of 37 ± 0.2°C. At appropriate intervals, aliquots of 100 μl were removed and immediately analyzed by high-performance liquid chromatography (HPLC). Pseudo-first-order rate constants for the hydrolysis of the derivatives were determined from the slopes of linear plots of the logarithms of residual compound against time. HPLC analyses were performed with a Waters Associates Model 600 pump equipped with a Waters 990 variable wavelength UV detector and a 20 μl loop injection valve. A reversed phase Symmetry C18 (25 cm × 3.9 mm; 5 μm particles) column in conjunction with a precolumn (Sentry Guard Symmetry C18, 20 × 3.9 mm) was eluted with mixtures of methanol and 50 mM acetate buffer pH 4.7 70/30 (v/v). The injected volume was 20 μl. The flow rate of 0.8 ml/min was maintained and the column effluent was monitored continuously at 254 nm. The compounds were estimated by measuring the peak areas or peak heights in relation to those of standards chromatographed under the same conditions.

Stability in Physiological Medium

The stability of Dopimid compounds **1**, **2**, **5**, **6**, **9**, **10** and L-Dopa in physiological medium was studied at 37°C in 0.05 M phosphate buffer saline (0.14 M NaCl) at pH 7.4, containing 50% v/v of rat serum. The stability of L-Dopa in physiological medium was carried out by adding 10 μl of a stock solution in phosphate buffer pH 8.0 (4.2 mg/ml) to 1.6 ml of the preheated serum solution. The final concentration was about 100 μM. The resulting test solutions were maintained at 37 ± 0.2°C in a shaker water bath. Aliquots of 100 μl were withdrawn at appropriate intervals and treated with 500 μl of cold acetonitrile in order to precipitate the serum proteins. After mixing and centrifugation for 5 min at 13,200 rpm, the clear supernatant (20 μl) was analyzed by HPLC. Pseudo-first-order rate constants for degradation of the derivatives were determined from the slopes of linear plots of the logarithms of residual dopamine or L-Dopa conjugate against time.

For assessment of L-Dopa stability a reversed phase Phenomenex Synergi Hydro C18 (25 cm × 3.9 mm; 5 mm particles) column in conjunction with a precolumn (Sentry Guard Symmetry C18, 20 × 3.9 mm) was used with 20 mM K_2HPO_4 buffer pH 2.5. The injected volume was 20 μl. The flow rate of 0.8 ml/min was maintained and the column effluent was monitored continuously at 280 nm.

Receptor Binding Assays

Adult male or female Sprague–Dawley CD rats (Charles River, Como, Italy) with body masses of 200–250 g at the beginning the experiments, were maintained under an artificial 12-h-light/dark cycle (light on 08.00–20.00 h) at a constant temperature of 23 ± 2°C and 65% humidity. Food and water were freely available, and the animals were acclimatized for >7 days before use. Experiments were performed between 08.00 and 14.00 h. Animal care and handling throughout the experimental procedure were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental protocol was approved by the Animal Ethical Committee of the University of Cagliari.

[^{3}H]Flunitrazepam Binding

Cerebral cortex was homogenized with a Polytron PT 10 in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and centrifuged twice at 20,000 × *g* for 10 min. The pellet was reconstituted in 50 volumes of Tris-HCl buffer and was used for the binding assay. Aliquots of 400 μl of tissue homogenate (0.4–0.5 mg of protein) were incubated in presence of [^{3}H]flunitrazepam (spec. act. 7.4 Ci/mmol, New England Nuclear) at a final concentration of 0.5 nM, in a total incubation volume of 1,000 μl. Drugs were dissolved in DMSO and serial dilutions were made up in DMSO and added in 100 μl aliquots. After a 60 min incubation at 0°C, the assay was determined by rapid filtration through glass-fiber filter strips (Whatman GF/B). The filters were rinsed with 2- to 4-ml portions of ice-cold Tris-HCl buffer as described above. Radioactivity bound to the filters was quantitated by liquid scintillation spectrometry (Ultima Gold, Camberra Packard). Nonspecific binding was determined as binding in the presence of 5 μm diazepam, and represented about 10% of total binding.

[^{3}H]PK 11195 Binding

After sacrifice brain and ovary were rapidly removed, cerebral cortex was dissected and both tissues were stored at −80°C until assay. The tissues were thawed and homogenized in 50 volumes of Dulbecco's phosphate buffered saline (PBS) pH 7.4 at 4°C with a Polytron PT 10 (setting 5, for 20s). The homogenate was centrifuged at $40{,}000 \times g$ for 30 min, and the pellet was resuspended in 50 volumes of PBS and recentrifugated. The new pellet was resuspended in 20 volumes of PBS and used for the assay. [^{3}H]PK 11195 binding was determined in a final volume of 1,000 µl tissue homogenate (0.15–0.20 mg protein), 100 µl of [^{3}H]PK 11195 (spec. act. 85.5 Ci/mmol, New England Nuclear) at final assay concentration of 1 nM, 5 µl of drug solution or solvent and 795 µl of PBS buffer (pH 7.4 at 25°C). Drugs were dissolved in dimethylsulfoxide and serial dilutions were made up in dimethylsulfoxide and added in 5 µl aliquots. Incubations (0°C) were initiated by addition of membranes and were terminated 90 min later by rapid filtration through glass-fiber filter strips (Whatman GF/B), which were rinsed with two 4 ml of ice-cold PBS buffer using Cell Harvaster filtration manifold (Brandel). Filter bound radioactivity was quantified by liquid scintillation spectrometry (Ultima Gold, Camberra Packard). Non specific binding was defined as binding in the presence of 10 µM unlabelled PK 11195 (Sigma).

DA D_2-like Receptor Binding Assay

Rats were killed by decapitation, and the striatum was immediately dissected on ice. The tissue was rapidly homogenized in 200 vol (200 ml per gram of wet tissue) of ice-cold 50 mM Tris-HCl (pH 7.7 at 25°C), designated buffer A, with the use of a Teflon pestle and glass homogenizer (two series of 15 strokes with a 1-min interval between series). The homogenate was centrifuged for 10 min at $43{,}000 \times g$, and the resulting pellet was resuspended and homogenized in 200 vol of buffer B (buffer A containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM EDTA, and 5.7 mM ascorbic acid) before use in the binding assay. The binding of [^{3}H]YM-09151 was determined in a final volume of 1 ml consisting of 400 µl of membrane suspension (1.5–2.0 mg of protein), 100 µl of 0.4 nM [^{3}H]YM-09151 (84 Ci/mmol; New England Nuclear), 5 µl of drug (10^{-11}–10^{-4} M, dissolved and diluted in DMSO) or solvent, and buffer B to volume. Nonspecific binding was determined in the presence of 50 µM L-sulpiride (Knoll S.p.A., Milan, Italy). After incubation for 60 min at 25°C in the dark, the binding reaction mixtures were passed through glass-fiber (Whatman GF/B) filters with the use of a Cell-Harvester filtration manifold (M24; Brandel, Gaithersburg, MD). Filters were then rinsed twice with 4 ml portions of ice-cold buffer A and transferred to vials containing 3.5 ml of scintillation cocktail (Ultima Gold, Camberra Packard). Radioactivity associated with the filters was quantified with a scintillation spectrometer.

Cell Culture

Madin–Darby Canine Kidney (i.e., MDCK) cells, retrovirally transfected with the human MDR1 cDNA (MDCKII-MDR1) was a gift from Professor Piet Borst (The Netherlands Cancer Institute, Amsterdam, The Netherlands). The growth medium Dulbecco's Modified Eagle Medium (DMEM), trypsin/EDTA solution, calf serum, minimum essential medium (MEM) and non-essential amino acids were obtained from Gibco (Invitrogen, Grand Island, NY, USA). Penicillin (10,000 U/ml), streptomycin (10,000 µg/ml), sodium bicarbonate, HEPES and quinidine (P-gp inhibitor) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [^{3}H]Ritonavir (specific activity 1.0 Ci/mmol) was obtained from Moravek Biochemicals (Brea, CA, USA). [^{3}H]Diazepam (specific activity 76 Ci/mmol), [^{14}C]mannitol (specific activity 55 mCi/mmol) and [^{3}H]L-Dopa (specific activity 50 Ci/mol) were purchased from Perkin Elmer Life Science Products (Boston, MA, USA) and from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA), respectively. Culture flasks (75 cm^2 growth area), polyester Transwells® (pore size 0.4 µm and 6.5 mm diameter) and 12 well plates were obtained from Corning Costar (Cambridge, MA, USA). Buffer components and other solvents were obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA).

Uptake Studies

Uptake studies were performed using confluent cells monolayer 6–8 days post seeding. Medium was aspirated and cells were washed thrice with Dulbecco's phosphate buffered saline (DPBS), pH 7.4. Uptake was initiated by adding 1 ml of drug solution at increasing concentration (10, 25, 50 and 75 µM, respectively), in DPBS 0.01% (v/v) Cremophor EL 5% (v/v) DMSO, in the presence or absence of competing substrates, to the wells. Incubation was carried out for a period of 10 min at 37°C in a humidified atmosphere with 5% CO_2. At the end of the incubation period drug solution was removed and the cells monolayer was washed thrice with ice-cold stop solution. Cells were lysed overnight by adding 1 ml 0.1% w/v Triton X-100 and 0.3 *N* sodium hydroxide at RT. Aliquots of 500 µl were withdrawn from each well and transferred to scintillation vials containing 5 ml scintillation cocktail. Samples were then analyzed by liquid scintillation spectrophotometry using a Beckman scintillation counter (Model LS-6500, Beckman Instruments, Inc.). Rate of uptake was normalized to the protein content of each well. Amount of protein in the cell lysate was quantified by the method of Bradford utilizing Bio-Rad protein estimation kit (Bio-Rad, Hercules, CA, USA).

All uptake experiments were performed at least in quadruplicate ($n = 4$) and results are expressed as mean ± SD. The permeability coefficient experiments were carried out in triplicate ($n = 3$) and results are expressed as mean ± SD. Statistical analyses were performed using Student's t test between two mean values. A probability of less than 0.05 ($p < 0.05$) was considered to be statistically significant.

Bi-directional Transport Studies on MDCKII-MDR1 Monolayers

Bi-directional transport studies were carried out using MDCKII-MDR1 cells monolayers grown on 12 well Transwell® insert (diameter 6.5 mm). All experiments were done at 37°C in air. Medium was aspirated from both the

apical (AP) and basolateral (BL) chambers of each insert and cells monolayers were washed three times (10 min per wash) with DPBS pH 7.4. The formation of confluent MDCKII-MDR1 monolayers with tight junctions was confirmed by microscopy and TEER values. [^{3}H]Diazepam and [^{13}C]mannitol were used as markers for the transcellular and paracellular pathway, respectively, and as an internal control to verify tight junction integrity during the assay. Test drug solution in DPBS 0.01% (v/v) Cremophor EL 5% (v/v) DMSO at a concentration of 100 μM was added to the donor side (0.5 ml for the AP chamber and 1.5 ml for the BL chamber) and fresh DPBS was placed in the receiver compartment. For AP-to-BL or BL-to-AP flux studies, the drug solution was added in the AP chamber or in the BL chamber, respectively. Aliquots (200 μl) were withdrawn from the receiver side at various time intervals of 180 min, and replaced with fresh DPBS pH 7.4 to maintain sink conditions. Samples were stored at −80°C until further analysis. Statistical analyses were performed using analysis of variance (ANOVA) followed by the Tukey post hoc tests (GraphPad Prism v. 4 for Windows, GraphPad Software, San Diego, CS). Differences were considered statistically significant at $p < 0.05$.

The apparent permeability coefficient (P_{app}) was calculated according to the following equation:

$$P_{app} = \frac{V \cdot dC}{A \cdot C_0 \cdot dt} \quad (1)$$

where $V \cdot (dC/dt)$ is the linear appearance rate of mass in the receiver solution, A is the filter/cell surface area and C_0 is the initial concentration of the test compounds. The net efflux of a test compound was assessed by calculating the ratio of P_{app} in the BL-to-AP direction vs. P_{app} in the AP-to-BL direction ($P_{app\ BL\text{-}to\text{-}AP}/P_{app\ AP\text{-}to\text{-}BL}$). A ratio of substantially greater than 1.0 indicates net efflux (13).

[^{3}H]Diazepam, [^{14}C]mannitol and [^{3}H]L-Dopa samples were analyzed by liquid scintillation spectrophotometry using a Beckman scintillation counter (Model LS-6500, Beckman Instruments, Inc.). Dopimid compounds **1**, **2**, **5**, **6**, **9**, **10**, and dopamine hydrochloride samples were assayed by HPLC. HPLC analyses were performed with a HP 1050 pump equipped with a Waters 990 variable wavelength UV detector and an Alcott autosampler (model 718AL HPLC). For transport studies on Dopimid compounds **1**, **2**, **5** and **6**, a reversed phase Phenomenex Synergi Hydro C_{18} (25 cm × 4.6 mm; 4 μm particles) column in conjunction with a precolumn (Sentry Guard Symmetry C_{18}, 20 × 3.9 mm) was used with mixtures of methanol and deionized water 70/30 (v/v). A 50 μl of the sample was injected and the flow rate of 0.8 ml/min was maintained. The column effluent was monitored continuously at 254 nm. Quantification of the compounds was carried out by measuring the peak areas in relation to those of chromatographic standards under the same conditions. Analyses of Dopimid compounds **9** and **10** were performed similarly with a change in mobile phase containing methanol and deionized water 30/70 (v/v). The flow rate of 0.6 ml/min was maintained and the column effluent was monitored continuously at 254 nm. Flux of dopamine hydrochloride was evaluated by using the reported HPLC conditions, except for the composition of elution mixture (20 mM phosphate buffer, pH 2.5), flow rate (1 ml/min) and wavelength ($\lambda = 280$ nm).

Computational Calculations of Physicochemical and Permeability Characteristics of Dopimid Compounds 1–12

Lipophilicity of the examined compounds was estimated by using C Log P (v. 4, BioByte Corp) software. To estimate the BBB penetration of Dopimid compounds **1–12**, their log C_{brain}/C_{blood} (log BB) values were calculated according to the equation: $\log BB = -0.0148(\pm 0.001)\,PSA + 0.152(\pm 0.036)\,C\log P + 0.139(\pm 0.073)$ (14) in which PSA is the polar surface area (defined as the surface area in Å^2 occupied by nitrogen and oxygen atoms and polar hydrogens connected to these heteroatoms) and Clog P is the calculated log Po/w (octanol/water partition coefficient). Calculation of the polar surface area was made by using a simple protocol proposed by Ertl *et al.*, (15) allowing the so-called topological PSA (TPSA). The lipophilicity indexes of Dopimid compounds **1–12**, expressed as $\log k' = \log (t_R - t_0)/t_0$, were obtained eluting these compounds on a reversed phase Symmetry C_{18} (25 cm × 3.9 mm; 5 μm particles) column in conjunction with a precolumn (Sentry Guard Symmetry C18, 20 × 3.9 mm) with mixtures of methanol and acetate buffer pH 4.7 70/30 (v/v). The injected volume was 20 μl. The flow rate was maintained at of 0.8 ml/min and the column effluent was monitored continuously at 254 nm. High-performance liquid chromatography (HPLC) analyses were performed with a Waters Associates Model 600 pump equipped with a Waters 990 variable wavelength UV detector. The column effluent was monitored continuously at 254 nm. HPLC mobile phase was prepared using HPLC-grade methanol.

Isolation and Culture of BBMEC

The bovine brain microvessel endothelial cells (i.e., BBMECs) were isolated from the gray matter of bovine brains following an isolation procedure described previously (16,17). Briefly two or more freshly excised bovine brains were purchased from Steve's Meat Market (DeSoto, KS, USA) and transported to the laboratory in ice-cold MEM. Subsequently, all the procedures for isolation were performed in aseptic condition using sterile materials and laminar flow hood. The large surface capillaries and meninges were removed from the brains and the gray matter was scraped off and minced into small pieces (1–2 mm). The gray matter suspension was treated with dispase for 30 min in the shaker bath set at 37°C and 100 oscillations per min. The pH of the suspension was adjusted to 7.4, by adding MEM pH 9–10. The suspension was returned to the same setting for an another hour. The suspension was then centrifuged at 3,200 rpm (Beckman centrifuge, rotor-JA 14) for 10 min at 4°C. Three distinct layers: a red pellet at the bottom, a large semisolid pinkish layer in the middle and brown liquid supernatant at the top were thus obtained. The brown liquid supernatant was discarded and the rest of the suspension was mixed with 13% complete dextran solution and centrifuged at 7,730 rpm (Beckman centrifuge, rotor-JA14) for 10 min. On centrifugation, a thick red pellet was obtained containing

erythrocytes and micro vessels. These micro vessels were further treated with collagenase/dispase for approx 2.5 h at shaker bath to separate the endothelial cells from the fat, myelin, and other cells. Micro vessel endothelial cells were collected from second layer of the percoll gradient, rinsed with MEM and stored in freezing medium containing 70% of MEM-F12 medium, 20% horse serum and 10% DMSO at −80°C and then in liquid nitrogen.

The isolated brain micro vessel endothelial cells were thawed at 37°C, rinsed and resuspended in culture medium containing 45% v/v MEM, 45% v/v Ham's F-12 nutrient mixture, 10% v/v horse serum, 100 μg/ml penicillin-G, 100 μg/ml streptomycin, 100 μg/ml heparin, 2.5 μg/ml amphoter icin-B, 50 μg/ml polymyxin-B and seeded on collagen-fibronectin coated 12 well plates at density of 50,000 cells cm^2. The cells were incubated at 37°C, 5% CO_2 and 95% humidity for 3–4 days undisturbed. After that the cells were fed with the aforementioned medium without amphotericin-B and polymyxin-B every other day until formation of a confluent monolayer. Cells were used for uptake studies usually after 12–14 days.

Co-culture of BBMECs and Astrocytes

In order to establish some of the cellular environment complexities that exist *in vivo*, we used an *in vitro* model of BBB by growing endothelial cells on one side of collagen-coated membrane inserts and astrocytes on the bottom of six well plastic dishes, as previously described (18,19). Human astrocytes were purchased from ATCC. These cells were tested negative for HIV-1 and hepatitis-B DNA by PCR. Cells were grown to confluence in 7–10 days in RPMI-1640 medium supplemented with 10 μg/ml human recombinant epidermal growth factor (hEGF), 10 mg/ml insulin, 50 μg/ml gentamicin, 50 mg/ml amphothericin-B and 5% v/v fetal bovine serum (FBS), in 75 cm^2 culture flasks at 37°C and a water saturated atmosphere containing 5% CO_2.

Astrocytes were plated, with a density of 50,000 cells/cm^2, on the under side of a collagen-coated polyester Transwell® membrane in the specific medium supplemented with all the factors described above. After 48 h, the astrocyte medium in the basal compartment was replaced. The medium in the apical chamber was removed and was replaced with the BBMECs specific medium, described above, supplemented with 0.5% v/v of endothelial growth factor (ECGF). BBMECs were seeded in the apical chamber at 50,000 cells/cm^2. The Transwell® plates were then incubated at 37°C in an atmosphere containing 5% CO_2. Under these experimental conditions, BBMECs formed a confluent monolayer within 15 days. Cell confluence was assessed by light microscopy and transepithelial electric resistance (TEER) and flux of [^{14}C]mannitol were measured as markers of cell monolayers integrity. TEER values of the cell monolayers were approximately 200 ohm·cm^2 and [^{14}C]mannitol transport was <0.5%/h in representative cell monolayers.

Transport Studies on BBMECs Monolayers

Permeability experiments were carried out using confluent BBMECs monolayers grown on 12 well Transwell® insert (diameter 6.5 mm). All experiments were done at 37°C in air. Medium was aspirated from both the apical AP and BL chambers of each insert and cells monolayers were washed three times (10 min per wash) with DPBS pH 7.4. These transport experiments were studied by using Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** at 100 μM and confluent BBMEC monolayers grown on 12 well Transwell® on one side of collagen-coated membrane inserts and astrocytes on the bottom of six well plastic dishes. Again [^{3}H]diazepam and [^{13}C]mannitol were used as markers for the transcellular and paracellular pathway, respectively, and as an internal control to verify tight junction integrity during the assay. [^{3}H]L-Dopa and dopamine hydrochloride were also included in transport experiments. The drug solution was added in the AP chamber at a concentration of 100 μM and fresh DPBS was placed in the BL chamber. Drug solutions were prepared in DPBS 0.01% (v/v) Cremophor EL 5% (v/v) DMSO. Volumes of the AP and the BL chambers were 0.5 and 1.5 ml, respectively. Aliquots (200 μl) were withdrawn from the receiver side at various time intervals to 180 min, and replaced with fresh DPBS pH 7.4 to maintain sink conditions. Samples were stored at −80°C until further analysis. The permeability coefficient (P_{app}) was determined according to Eq. 1 as described above. Samples collected were analyzed by HPLC or counted on a liquid scintillation counter under the same conditions as illustrated previously for transport studies on MDCKII-MDR1 cells monolayers.

Brain Microdialysis

Dopimid compounds **5**, **6**, **9** were dissolved in distilled water and injected intraperitoneally in a volume of 3 ml/kg of body mass. Rats were anesthetized with chloral hydrate (0.4 g/kg, i.p.) and a concentric dialysis probe was inserted at the level of the medial prefrontal cortex (A + 3.2, ML + 0.8, V −5.3 relative to the bregma) according to the Paxinos atlas (20). The active length of the dialysis membrane (Hospal Dasco, Bologna, Italy) was restricted to 2 mm.

Experiments were performed ~24 h after probe implantation, as previously described (21). Samples were collected every 20 min for all the experiments. The detection limit for dopamine was 2 fmol per injection. The average neurotransmitter concentration in the last three samples before treatment was taken as 100%, and all posttreatment values were expressed as means ± SEM relative to the basal value. The mean *in vitro* recovery of the probes was 24 ± 3%. All probes were tested before implantation, and those with a recovery value outside of this range were not used. The absolute concentration of dopamine was not corrected for this value. At the end of each experiment, the placement of the probe was verified histologically. All rats in which the probe was located outside of the prefrontal cortex were excluded from the analysis. Data of microdialysis studies are presented as means ± SEM. Comparisons among groups were performed by two-way analysis of variance (ANOVA) for repeated measures, with factors being treatment and time points. For statistical analysis, the raw baseline values of dopamine concentration were used. Absolute basal dopamine concentrations are reported in the Results section. Post hoc comparisons were performed by Neuman–Keuls test. A p value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Synthesis of Dopimid Compounds 1–12

As reported in Fig. 1, two methods were employed to prepare the compounds **16 a–d**. The Method A involving at first the condensation of the appropriate imidazopyridinacetic acid **15 a–d** (10) with 3,4-bis(tert-butyldimethylsilyloxy)-L-Dopa ethyl ester **14** in anhydrous THF and in the presence of EEDQ as dehydrating agent leading to the corresponding compounds **16 a–d**. Removal of the silyl groups of compounds **16 a–d** to obtain the corresponding Dopimid compounds **5–8** was accomplished by using TFA. Compounds **5–8** were also prepared in a straightforward fashion by condensation of the appropriate imidazopyridinacetic acid **15 a–d** with the ethyl ester hydrochloride **13** in anhydrous DMF and in the presence of CDI (Method B). By these methods, Dopimid compounds **5–8** were obtained in moderate to good yields. However, in terms of step's number and yields, Method B proved to be overall more advantageous. The esters **5–8** were subsequently hydrolysed with HCl 1*N* in dioxane to give the corresponding carboxylic acids **1–4**. As for the synthesis of dopamine derivatives **9–12** (Fig. 2), it was accomplished by condensation of the required imidazopyridinacetic acid **15 a–d** with the *O*-silyl protected dopamine **18** to give the silylated compounds **19 a–d**. Removal of their *O*-silyl groups by TFA readily afforded the dopamine derivatives **9–12**. All the compounds were characterized by IR, ^{1}H NMR and mass spectroscopy. Table I reports yields and melting points of the compounds **1–12**, **16 a–d** and **19 a–d**.

Stability Studies

The hydrolysis rate of representative Dopimid compounds such as **1**, **2**, **5**, **6**, **9**, and **10** was examined in 0.05 M phosphate buffer at pH 7.4 and in diluted rat serum solution both at 37°C. Pseudo first-order kinetics were always observed and the half-lives of starting materials computed (Table II). They were found in the range 11–30 h when phosphate buffer was used while, in dilute rat serum, the half-lives were in the ranges 18–20 min and 2.42–2.96 h for **5**, **6** and **1**, **2**, **9**, **10**, respectively. It should be noted that L-Dopa decomposition similarly occurred, with a half-life of 1 h (Table II). To gain information on the degradation pathway, the main degradation products of the representative Dopimid compounds **1**, **5**, and **9** in physiological medium were characterized by LC-mass spectrometry (LC-MS). Thus, the ESI LC-MS analysis in negative mode of the mixture from degradation of **1** in physiological medium after 2 h showed the presence of a peak at m/z 532 attributable to $[M-H]^-$ and another peak at m/z 309 attributable to imidazopyridinmethyl ion. By ESI LC-MS analysis in negative mode of the mixture obtained from the stability studies in physiological medium of compound **5** it was detected after 30 min the presence of the compound **1** $[M-H]^-$ (m/z 532) and traces of the imidazopyridinmethyl ion (m/z 309). In the case of **9**, under analogous conditions and only after 2 h, ESI LC-MS analysis revealed the peak attributable to the imidazopyridinmethyl ion (m/z 309) together with the one of the intact compound **9** $[M-H]^-$ (m/z 488). Therefore, all of these results suggest that in physiological medium, the ester compound **5** undergoes a rapid cleavage to give the corresponding acid **1** which, as the dopamine derivative **9**, is hydrolyzed at the amide bond level leading to the corresponding imidazopyridinacetic acid **15a**.

Radioligand Binding Assays

$GABA_A$ receptors are allosterically modulated by several types of compounds among which the benzodiazepines (22). Moreover, there is overwhelming evidence that binding sites of benzodiazepines can be located both at central

Table II. Stability Data in Phosphate Buffer and Diluted Rat Serum, Physicochemical Parameters and Estimation of BBB Penetration for Dopimid Compounds **1–12**

Comp.	CLOG P[a]	RP-HPLC log k'[b]	TPSA[b] (Å2)	Log BB[c]	$t_{1/2}$ (h) in 0.05M Phosphate Buffer (pH 7.4)	$t_{1/2}$ in 50% Lyophilized Rat Serum
1	4.46	−0.135	124	−1.02	19.5 ± 0.3 [e]	2.9 ± 0.2 (h) [d]
2	3.75	−0.271	124	−1.13	31.0 ± 1.4 [e]	3.0 ± 0.3 (h) [d]
3	3.75	−0.223	124	−1.12		
4	3.03	−0.522	124	−1.24		
5	5.13	−0.122	113	−0.76	18.9 ± 4.3 [d]	18.2 ± 1.8 (min) [d]
6	4.42	−0.271	443	−0.86	23.6 ± 5.7 [e]	20.4 ± 1.8 (min) [d]
7	4.42	−0.260	113	−0.86		
8	3.70	−0.393	113	−0.97		
9	4.53	−0.155	87	−0.46	11.1 ± 2.6 [d]	2.6 ± 0.4 (h) [d]
10	3.81	−0.292	87	−0.57	24.3 ± 6.3 [e]	2.4 ± 0.4 (h) [d]
11	3.81	−0.264	87	−0.57		
12	3.54	−0.485	87	−0.61		
L-Dopa						60.0 ± 1.2 (min) [e]

[a] Calculated according to CLOG P software.
[b] Calculated according to Ertl's method (15).
[c] Calculated according to Clark's model and by using TPSA (14).
[d] Data are means ± SD of three separate experiments.
[e] Data are means ± SD of two separate experiments.

(CBR) and peripheral (PBR) level (23) and the pharmacological effects mediated by these two receptor subtypes are very different. Alpidem interacts with both CBRs and PBRs. Previous works from these laboratories (24,25) pointed out that substitution of the alpidem at position 8 with lipophilic substituents and the presence of one chlorine atom at the *para* position of the phenyl ring at C(2) are key structural features for high binding affinity and selectivity toward PBRs.

The binding of Dopimid compounds **1–12** to the CBR and PBR receptors was evaluated by measuring their ability to inhibit [^{3}H]flunitrazepam and [^{3}H]PK 11195 (26) binding to membrane preparations arising from the cerebral cortex. Their effects were compared with those of unlabelled flunitrazepam and PK 11195, a selective ligand for PBRs (26). The affinities of compounds **1–12** to D_2-like receptors was measured by binding assays *in vitro* by using [^{3}H]YM-09151 as radioligand and membrane preparations from cerebral (striatum) homogenate. The inhibitory concentration (IC_{50}) values determined are listed in Table III together with that of the atypical antipsychotic clozapine for comparison.

As can be seen, Dopimid compounds **1–12** displayed no or low affinity for both CBR and PBR. Only compounds **5**, **9** and **10**, **12** possess a submicromolar binding affinity towards CBR and PBR subtypes, respectively. None of examined compounds showed affinity for dopaminergic receptors. Moreover, these binding results suggest that compounds **1–12** could be considered as inactive (or poorly active) derivatives of the active compound (i.e., L-Dopa or dopamine) and this is the requisite of a true prodrug as proposed by Albert (27).

Table III. Binding Data for CBR, PBR and DA Receptors of Dopimid Compounds **1–12**

Compound	IC_{50} (nM) [a,b]		
	CBR	PBR	[^{3}H] YM 09151
1	(24%)	>10,000	>10,000
2	(74%)	>10,000	>10,000
3	(13%)	>10,000	>10,000
4	(45%)	>10,000	>10,000
5	(44%)	327	>10,000
6	>10,000	>10,000	>10,000
7	(55%)	4,760	>10,000
8	(49%)	>10,000	>10,000
9	>10,000	165	>10,000
10	381.5	>10,000	>10,000
11	>10,000	9,776	>10,000
12	225.4	5,435	>10,000
flunitrazepam	5	–	–
PK 11195	–	4.27	–
clozapine	–	–	4,119

[a] Data are means ± SD of three separate experiments performed in duplicate.
[b] Values in parentheses are the percentages of inhibition of specific [^{3}H]flunitrazepam binding determined at 40 μM concentration of the tested compound for CBR affinity.

[^{3}H] Ritonavir Uptake and Bi-directional Transport Studies on MDCKII-MDR1 Cells

MDCK cell lines, as Caco-2 cells, are known to express P-glycoprotein (P-gp) which plays an important role in the efflux transport of drugs from the brain-to-blood (28). Therefore, the design of drugs that are not P-gp substrates is considered a valuable approach for obtaining higher BBB permeability (29). Thus, we were particularly interested to see if Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** are substrates of P-gp. To this purpose, at first a study aimed at evaluating the effect of the L-Dopa and dopamine derivatives on the uptake characteristics of a P-gp model substrate such as the [^{3}H]ritonavir was carried out by using MDCKII-MDR1 cells which are characterized by a high expression of P-gp (13). The formation of confluent MDCK monolayers with functional tight junctions was confirmed by microscopy, TEER values and flux of [^{14}C]mannitol. The average MDCK TEER value during this study was approximately 250 ohm·cm^2 and [^{14}C]mannitol transport was <0.5%/h in representative cell monolayers. The uptake experiments were performed by adding 1 ml of a [^{3}H] ritonavir solution (0.5 μCi/ml) to drug solution at increasing concentration (10, 25, 50 and 75 μM, respectively). Incubation was carried out for a period of 10 min. In these tests the known P-gp substrate quinidine, L-Dopa and dopamine hydrochloride were also included and the results obtained are shown in Fig. 3. As can be seen, Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** or L-Dopa or dopamine hydrochloride, exhibited no inhibitory effect on the [^{3}H]ritonavir uptake, while [^{3}H]ritonavir uptake increased (about 50%), in the presence of quinidine. These data indicate that compounds **1**, **2**, **5**, **6**, **9**, and **10** are not substrates of P-gp.

Next, to further support that compounds **1**, **2**, **5**, **6**, **9**, and **10** are not P-gp substrates, transport studies involving these compounds were performed on MDCK cells. Transport studies were conducted both in AP-to-BL and BL-to-AP direction and the results are reported in Table IV. Given that Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** showed not significant differences in P_{app} values between AP-to-BL and BL-to-AP direction, it constitutes further evidence that these compounds are not substrates of P-gp.

Computational Calculations of Physicochemical and Permeability Characteristics of Dopimid Compounds 1–12

To be effective as therapeutic agents, compounds acting at CNS level should possess optimal BBB penetration properties. These are related to molecular lipophilicity indexes such as the partition coefficient (log P) and capacity factor log k' (15,30). Therefore, the log P values of Dopimid compounds **1–12** when distributed between *n*-octanol and water were calculated by using the C log P computer program and the log k' values of these compounds estimated by measuring their retention times in RP-HPLC (Table II). The obtained partition coefficients and capacity factors are correlated by a linear relationship with acceptable statistics ($n = 12$, $r^2 = 0.71$, $s = 0.07$). Log *P* value of 2.1 is generally considered optimal for penetration of the BBB (31). As can be seen from Table II, compounds **1–12** possess log

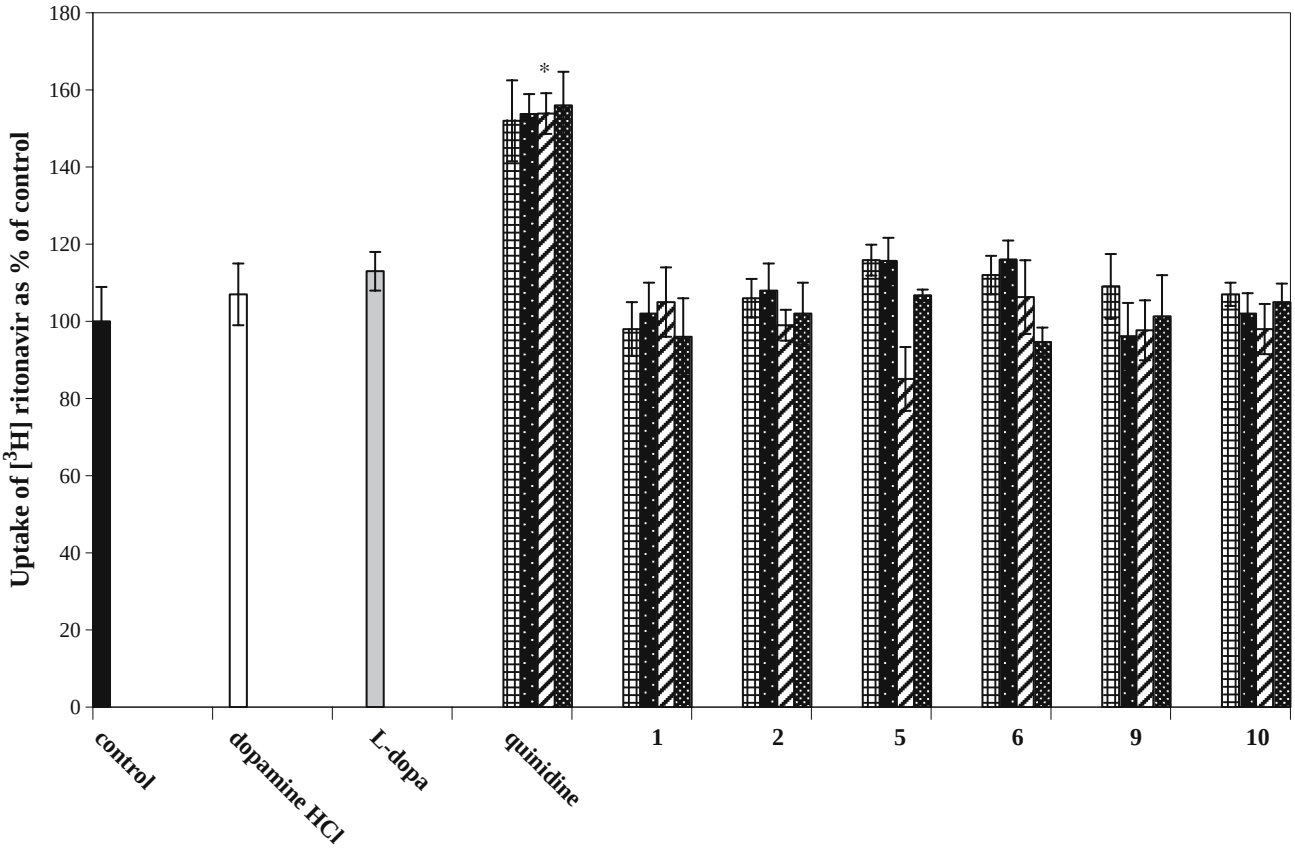


Fig. 3. Effect of Dopimid compounds **1**, **2**, **5**, **6**, **9**, **10**, quinidine, L-Dopa and dopamine hydrochloride on the [^{3}H]ritonavir uptake. The *bars* represent the uptake of [^{3}H]ritonavir as percent of control in the presence of the tested compounds.

P values in the range 3.03–5.13 and hence are lipophilic enough to cross the BBB. However, it has been pointed out that the log P is an important factor, although it correlates poorly with the log C_{brain}/C_{blood} (log BB) which is a more appropriate measure of the degree of BBB penetration. To estimate BBB penetration by compounds **1–12**, we used Clark's model (14) which relates log BB to polar surface area (PSA). This last was computed with a fragment-based approach, known as topological PSA (TPSA) approach (15). It is generally accepted that compounds with log BB > 0.3 cross the BBB readily, while compounds with log BB < −1.0 are poorly distributed to the brain. As can be seen from the data reported in Table II, Dopimid compounds **5**, **6**, **9** and **10** but not **1–4** should be able to cross the BBB.

Transport Across Co-cultures of BBMECs and Astrocytes Monolayers

To obtain more accurate information on the ability of Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** to cross the BBB,

Table IV. Bi-directional Transport Across MDCKII-MDR1 Cells on Dopimid Compounds **1**,**2**,**5**,**6**,**9**, and **10**

Compound	$P_{app} \times 10^{-6}$ (cm/s)[a]		Efflux Ratio P_{app} (BA)/(AB)
	Apical-Basolateral	Basolateral-Apical	
1	0.36 ± 0.034	0.38 ± 0.026	1.05
2	0.38 ± 0.022	0.24 ± 0.045	0.63
5	1.91 ± 0.35	1.49 ± 0.19	0.78
6	2.26 ± 0.29	2.10 ± 0.22	0.93
9	1.47 ± 0.32	1.78 ± 0.52	1.21
10	1.24 ± 0.95	1.96 ± 0.26	1.58
Dopamine	0.39 ± 0.24	–	–
[^{3}H]L-Dopa	1.49 ± 0.36	–	–
Mannitol	0.19 ± 0.18	–	–
Diazepam	2.30 ± 0.21	–	–

[a] Data are means ± SD of three determination.

Table V. Transport Across BBMEC on Dopimid Compounds **1**,**2**,**5**,**6**,**9**, and **10**

Compound	$P_{app} \times 10^{-6}$ (cm/s)[a]
1	0.33 ± 0.44
2	0.21 ± 0.10
5	2.77 ± 0.21
6	2.10 ± 0.31
9	3.86 ± 0.38*
10	2.98 ± 0.19*
Dopamine	0.21 ± 0.28
[^{3}H]L-Dopa	1.92 ± 0.14
Mannitol	0.19 ± 0.14
Diazepam	1.72 ± 0.09

[a] Data are means ± SD of at least three determinations.
*$p < 0.001$ vs dopamine.

transport studies involving co-culture of BBMECs and astrocytes were carried out. This approach constitute a well established *in vitro* method to estimate compound permeability across the BBB (32). The results indicate that dopamine derivatives **9** and **10** are characterized by P_{app} values greater than dopamine hydrochloride (up to tenfold, Table V). As expected, the esters **5** and **6** showed BBMEC monolayers permeability greater than L-Dopa which, in turn, exhibited a transport greater than dopamine hydrochloride. The rank order of P_{app} values observed in these assays was **9, 10** > **5** ≃ **6** ≃ L-Dopa >>**1** and **2** = dopamine (Table V). This trend parallels the rank order of BBB penetration calculated by the computational approach (Table II).

Microdialysis Studies

Microdialysis is a well-established sampling method for determining neurotransmitter concentrations in the brains of freely-moving animals (33). In this work, we decided to use such a very sensitive analytical methodology for determining the dopamine levels following the intraperitoneal (i.p.) administration of compounds **5**, **6**, and **9** to gain complete evidence for a prodrug-type mechanism. As shown in Fig. 4, the acute administration of compound **5** (10–20 mg/kg, i.p.) induced a dose-dependent increase in cortical dopamine output. The maximal increase (+197%), obtained with the dose of 20 mg/kg, was observed 120 min after drug injection and persisted for more than 240 min. ANOVA revealed a significant main effect of treatment [$F(3,111) = 4.369$; $p < 0.001$]; a significant main effect of repeated measures [$F(6,111) = 1.369$; $p < 0.001$]; and a significant interaction between factors [$F(12,111) = 0.259$; $p < 0.001$].

The acute administration of compound **6** (10–20 mg/kg, i.p.) induced a smaller and less persistent effect on cortical dopamine output. The maximal increase (+90% over basal values) was observed 40 min after the injection of the drug

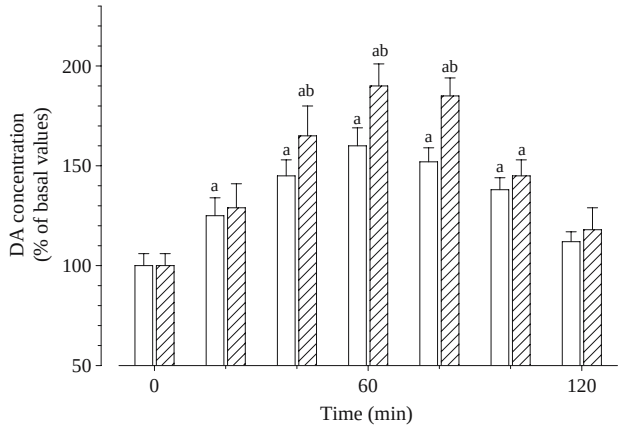


Fig. 5. Effect of acute administration of compound **6** on cortical extracellular concentration of dopamine in the rat medial prefrontal cortex. The drug has been administered intraperitoneally at the dose of 10 (*open bars*) or 20 (*hatched bars*) mg/kg. Data are means ± SEM of at least five rats per dose, are expressed as a percentage of basal values and represent the samples collected every 20 min after drug administration. [a]$p < 0.01$ vs basal values; [b]$p < 0.05$, [b′]$p < 0.01$ vs previous dose.

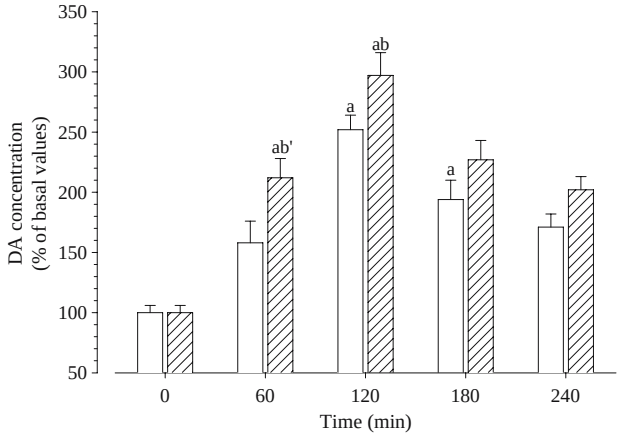


Fig. 4. Effect of acute administration of compound **5** on cortical concentration of dopamine in the rat medial prefrontal cortex. The drug has been administered intraperitoneally at the dose of 10 (*open bars*) or 20 (*hatched bars*) mg/kg. Data are means ± SEM of at least five rats per dose, are expressed as a percentage of basal values and represent the samples collected every hour after drug administration. [a]$p < 0.01$ vs basal values; [b]$p < 0.05$, [b′]$p < 0.01$ vs previous dose.

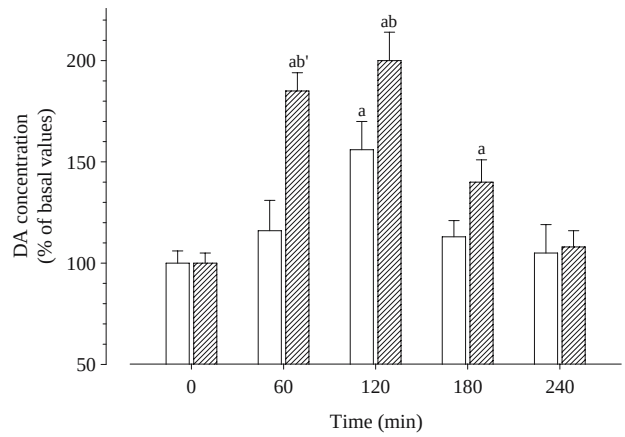


Fig. 6. Effect of acute administration of compound **9** on cortical concentration of dopamine in the rat medial prefrontal cortex. The drug has been administered intraperitoneally at the dose of 10 (*open bars*) or 20 (*hatched bars*) mg/kg. Data are means ± SEM of at least five rats per dose, are expressed as a percentage of basal values and represent the samples collected every hour after drug administration. [a]$p < 0.01$ vs basal values; [b]$p < 0.05$, [b′]$p < 0.01$ vs previous dose.

and returned to basal values in 80 min. ANOVA revealed a significant main effect of treatment [$F(3,111) = 12.345$; $p < 0.001$]; a significant main effect of repeated measures [$F(6,111) = 0.957$; $p < 0.001$]; and a significant interaction between factors [$F(12,111) = 1.520$; $p < 0.001$] (Fig. 5).

Acute administration of compound **9** (10–20 mg/kg, i.p.) elicited a dose- and time-dependent increase in the concentration of dopamine in the rat medial prefrontal cortex. This increase was maximal (~100% over basal values) 120 min after drug administration and returned to basal values in 240 min (Fig. 6). ANOVA revealed a significant main effect of treatment [$F(3,111) = 58.596$; $p < 0.001$]; a significant main effect of repeated measures [$F(6,111) = 2.532$; $p < 0.001$]; and a significant interaction between factors [$F(12,111) = 2.486$; $p < 0.001$]. For Figs. 4 and 6 as the effect of the compounds tested was persistent, only the data collected at 60, 120, 180 and 240 min were reported.

Our microdialysis studies show that the compounds tested induce a dose- and time-dependent increase in the dopamine levels in the rat medial prefrontal cortex. Our results are very similar to those observed by Eltayb *et al.*, (34), who have shown that intraperitoneal administration of L-Dopa (3 mg/kg), in association with the peripheral decarboxylase inhibitor benserazide (25 mg/kg), induced a marked (+400%) and long lasting (>300 min) increase in cortical dopamine concentration measured with microdialysis.

The precise mechanism through which the prodrugs exert their pharmacological effect is unclear. It is possible that, once reached the brain, they release dopamine or L-Dopa, respectively, which in turn will increase the endogenous levels of catecholamines. While conversion of **9** in dopamine would directly increase endogenous dopamine levels, the action of **5** and **6** would be indirect. In fact, L-Dopa released in the extracellular space has to be transported into the presynaptic terminal where can be converted in dopamine by the aromatic amino acids decarboxylase, and, after its accumulation in the vesicles, released after depolarization of the presynaptic membrane.

CONCLUSION

In conclusion, in this study novel potential L-Dopa- and dopamine-prodrugs **1–12** were synthesized by linking these neurotransmitters to an appropriate 2-phenyl-imidazopyridine portion for testing purposes. Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** were found adequately stable against chemical hydrolysis in solution buffered at pH 7.4. Conversely, they undergo faster cleavage in dilute rat serum at 37°C. The analysis of the binding affinities of compounds **1–12** showed that they are essentially devoid of affinity for dopaminergic and benzodiazepine receptors. [^{3}H]Ritonavir uptake experiments by using MDCKII-MDR1 cells as well as transport studies on confluent MDCKII-MDR1 monolayers with tight junctions demonstrated that Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** are not substrates of P-gp. Computational calculations indicated a significant brain penetration for compounds **5–8** and **9–12**. Furthermore, transport studies involving co-cultures of BBMECs and astrocytes showed that Dopimid compounds **1**, **2**, **5**, **6**, **9**, and **10** should be able to cross the BBB. Brain microdialysis experiments showed that compounds **5**, **6**, and **9** induce a dose- and time-dependent increase in the dopamine levels in the rat medial prefrontal cortex. These results, taken together, lead to the conclusion that Dopimid compounds **5**, **6**, and **9** may be considered as novel potential L-Dopa and dopamine prodrugs.

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