Bioorganic & Medicinal Chemistry Letters 20 (2010) 2888–2891

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design of new dopamine D2 receptor ligands: Biosynthesis and pharmacological evaluation of the hydroxylated metabolite of LASSBio-581

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ARTICLE INFO

Article history:
Received 17 February 2010
Revised 3 March 2010
Accepted 5 March 2010
Available online 10 March 2010

Keywords:
Dopamine D2 receptor ligands
LASSBio-581
Antipsychotic prototype
Microbial hydroxylation
Biosynthesis
Cunninghamella echinulata

ABSTRACT

LASSBio-581 is a *N*-phenylpiperazine derivative designed for the treatment of schizophrenia. In this study, four strains of filamentous fungi were screened for their capabilities to biotransform LASSBio-581. *Cunninghamella echinulata* ATCC 9244 was chosen to scale up the biosynthesis of the *p*-hydroxylated metabolite of LASSBio-581. The chemical structure of the metabolite was confirmed by NMR, LC–MS and X-ray crystallography. Binding studies performed on brain homogenate indicated that the *p*-hydroxylated metabolite can be considered more selective for dopamine receptors than LASSBio-581, and, therefore, can be used to design new selective dopamine inhibitors.

LASSBio-581 is a new neuroactive compound designed and synthesized by applying molecular hybridization approach on the lead compounds clozapine (**1**) and L-741 (**2**) (Fig. 1).[1] The atypical antipsychotic agent, clozapine, effectively controls the positive and some of the negative symptoms of schizophrenia by binding to dopamine D4 and 5-HT_2 serotonin receptors.[2] However, this drug presents important hematological side effects, such as agranulocytosis, that restrict its use to those patients who do not respond to traditional therapy.[3] For this reason, the search for more efficient dopaminergic agents that have lower adverse effects is still an active research field. The *N*-phenylpiperazine derivative, LASSBio-581 (**3**), was designed as a selective ligand of dopamine D2 receptor with agonist activity modulated by chlorine atom in the aromatic ring and hypothermic action in assays with apomorphine in mice. The mechanism of action through the serotonergic neurotransmitter system was observed in subsequent evaluation.[3] Pharmacokinetics studies of LASSBio-581 have been performed in rats.[4] This previous study showed that LASSBio-581 is absorbed by oral and intra-peritoneal routes of administration, showing a two-phase pharmacokinetic disposition, but metabolites weren't identified.[4]

The use of microorganisms as models of mammalian metabolism was introduced in the early 1970s[5] and it's a very interesting tool in the production of molecules with improved, different or less toxic activity originated by the fungal enzymatic biodiversity. In recent years, our laboratory has carried out a number of studies on microbial models of mammalian metabolism.[6] Moreover, a number of studies have shown that filamentous fungi, particularly *Cunninghamella echinulata* and *Mortierella isabelina*, possess cytochrome P450 monooxygenase systems analogous to those in mammals.[7]

The *p*-hydroxylation seems to be a very common pathway for metabolism of LASSBio-581,[4] thus the aim of the present work was to use filamentous fungi to biosynthesize large amounts of the *p*-hydroxylated metabolite of LASSBio-581 (**4**) and to perform pharmacological evaluation of **4**. The pharmacological activity of the *p*-hydroxylated metabolite (**4**) biosynthesized was assayed for binding to serotonin and dopamine receptors.

Ten Erlenmeyer flasks containing 100 mL of liquid medium PDSM inoculated with 0.5 mL of a spore suspension of four different filamentous fungi strains obtained from 7 days grown potato agar slants and glycerol 25%. The flasks were incubated with LASSBio-581 dissolved in a solution of ethanol/dimethylformamide

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doi:10.1016/j.bmcl.2010.03.034

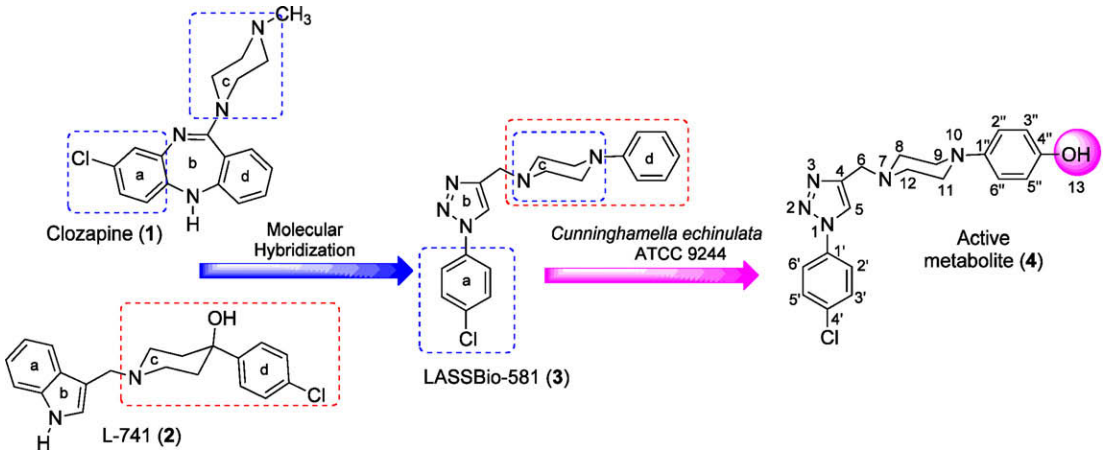

Fig. 1. Structural design concept of LASSBio-581 and its *p*-hydroxylated metabolite.

Table 1
^{1}H NMR data for LASSBio-581 and its metabolite **4** in MeOH-d_4 at 500 MHz (units: ppm)

Position	LASSBio-581	Metabolite **4**
5	δ = 7.95 (1H, s)	δ = 8.70 (1H, s)
6	δ = 3.83 (2H, s)	δ = 3.31 (s, 2H)
8, 12	δ = 2.72–2.77 (4H, m)	δ = 3.21–3.30 (4H, m)
9, 11	δ = 3.20–3.25 (4H, m)	δ = 3.21–3.30 (4H, m)
2′, 6′	δ = 7.71 (d, 2H, J = 8.7 Hz)	δ = 7.90 (d, 2H, J = 8.5 Hz)
3′, 5′	δ = 7.50 (d, 2H, J = 8,7 Hz)	δ = 7.62 (d, 2H, J = 8.5 Hz)
2″, 6″	δ = 6.82–6.95 (m, 2H)	δ = 6.2 (d, 2H, J = 8.7 Hz)
3″, 5″	δ = 7.22–7.30 (m, 2H)	δ = 6.90 (d, 2H, J = 8.7 Hz)
4″	δ = 6.82–6.95 (m, 1H)	—
13	—	δ = 4.29 (1H, s, OH)

(1:1) at a final concentration of 50 mg/100 mL. The flasks were kept in shaker under 200 rpm at 27 ± 2 °C for 60–65 h. Aliquots (1.0 mL) of the supernatant were taken every 24 h, up to 96 h and analyzed by HPLC. Control flasks consisted of culture broth without substrate to exclude components of cells walls fungi possibly detected by HPLC. The experiments without microorganisms were carried out to verify the stability of the substrate by addition of 1 mL of a solution of ethanol/ dimethylformamide (1:1). No oxidation products could be observed under these conditions. At the end of the process, the incubation medium was extracted with ethyl acetate to give oily crude which was purified by silica gel column chromatography using ethyl acetate/methanol (50:50, 70:30 and/or 95:05) as eluent. Final purifications were achieved by recrystallization. The compound obtained was characterized by NMR, LC–MS and X-ray crystallography.

The *p*-hydroxylated metabolite of LASSBio-581 (**4**) was obtained from biotransformation of LASSBio-581 by incubation with *C. echinulata* ATCC 9244, *C. echinulata* ATCC 9245, *C. echinulata* ATCC 36112 and *M. isabelina* NRRL 1757. Besides **4**, a variety of others metabolites was observed, essentially depending on the strain used. *C. echinulata* ATCC 9244 produced the major quantity of metabolites, therefore, was selected for the preparative-scale biotransformation of LASSBio-581. After 72 h of incubation, LASSBio-581 disappeared of the surnageant and *C. echinulata* ATCC 9244 produced basically the metabolite (**4**) at high concentrations in 24 h. Compound **4** was obtained after recrystallization as white crystals, yield of 62.5%, melting point 78.1 °C. ^{1}H NMR data of LASSBio-581 and **4** are shown in Table 1.

The structure of **4** was elucidated by the presence of OH peak at 4.29 δ and was correlated to the multiplet signal at position 4″ of

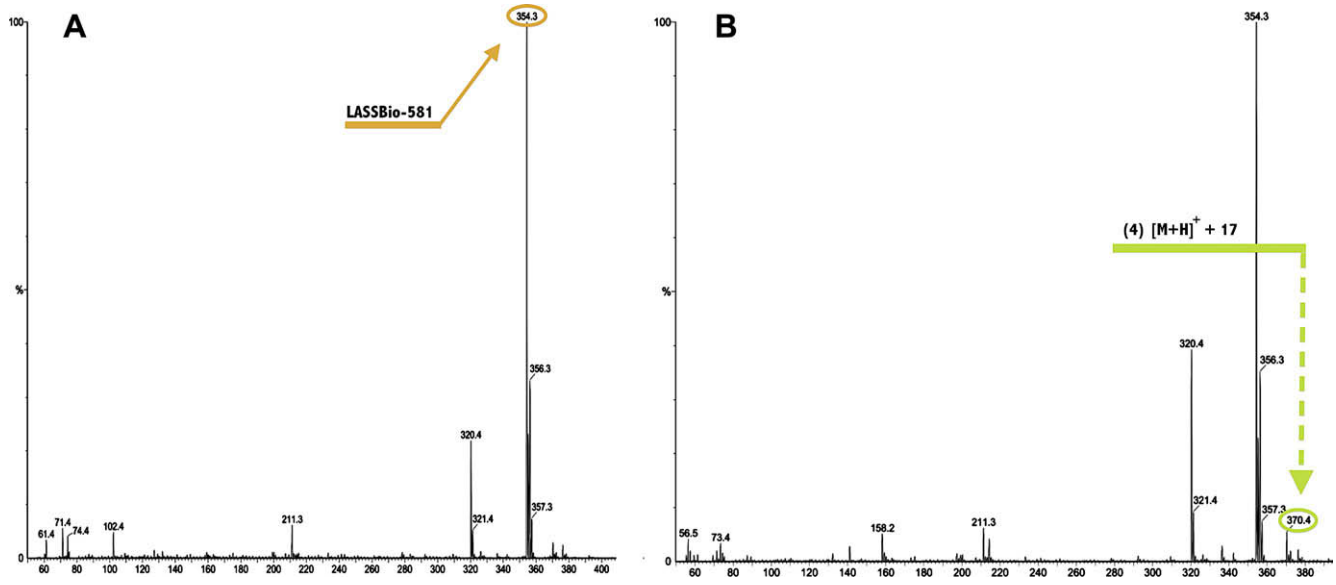

Fig. 2. LC–MS spectra of LASSBio-581 *m*/*z* 354.3 (A) and its *p*-hydroxylated metabolite (**4**) *m*/*z* 370.4 (B).

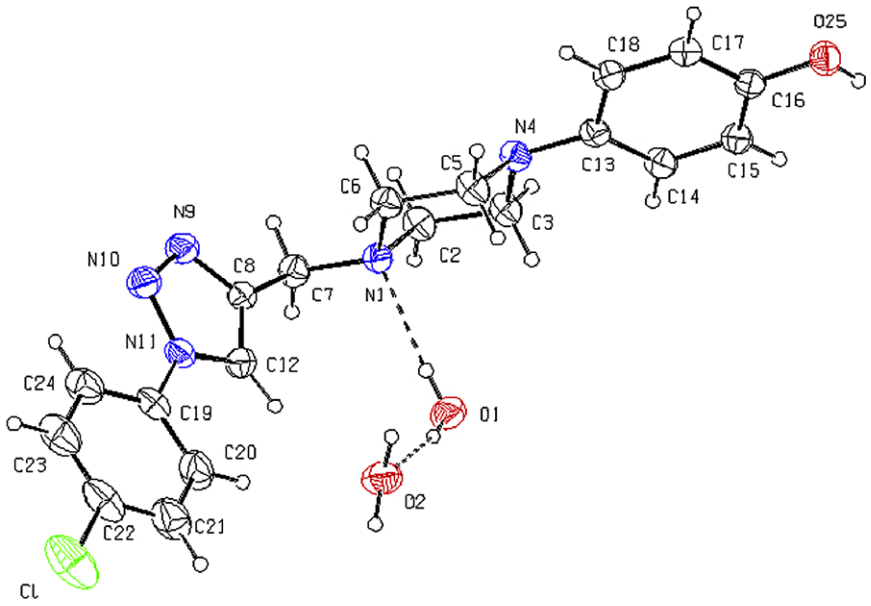

Fig. 3. X-ray structure of 4-(4-{[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-1-piperazinyl) phenol derivative of the LASSBio-581, that is, the *p*-hydroxylated metabolite (**4**).

LASSBio-581 (δ = 6.82–6.95). The mass of compound **4** was confirmed by LC–MS as *m/z* 370.4 for $[M+H]^+$ + 17 (OH) (Fig. 2). From the LC–MS analysis can be suggested that the product was modified without the loss of chlorine atom. From these spectral data, compound **4** was characterized as 4′′-hydroxylated.

The X-ray analysis of **4** confirmed the structure ascribed on the basis of the others spectra. The crystal structure of **4** is shown in Figure 3. Two water molecules fill the solvent cavity, both are involved in trifurcated strong intermolecular H-bonds of types O1—H1A...N1, O1—H1B...O2, O2—H2A...O25^{i}, O2—H2B...N9^{ii}, O25—H25...O1^{iii}, the symmetry operations and H-bonding geometry are listed in Table 2. Full list of atomic coordinates, thermal parameters, bond lengths and angles were deposited at the Cambridge Structure Database with CCDC code 746537.

The affinities of LASSBio-581 and its *p*-hydroxylated metabolite (**4**) for D_2-*like* receptors were determined via standard competition assays using rat striatum membranes with [^{3}H]-YM-9151-2 (nemonapride) as the radioactive ligand; their affinities for 5-HT_{1A} and 5-HT_{2A} receptors were determined using rat hippocampus membranes with [^{3}H]-8-OH-DPAT and rat cortex membranes with [^{3}H]-ketanserin, respectively, as detailed previously.[8]

As we can see from Table 3, the *N*-phenylpiperazine prototype LASSBio-581 binds with a moderate affinity to D_2-like, 5-HT_{1A} and 5-HT_{2A} receptors, presenting K_i values in the micromolar range. Its *p*-hydroxylated metabolite, **4**, maintains a similar moderate affinity for D_2-like receptors while its affinity for 5-HT receptors decreased (higher K_i values). The introduction of a hydroxyl group in the LASSBio-581 molecular scaffold resulted in a 6.5-fold decrease in 5-HT_{1A} affinity whereas no estimation of K_i was possible for 5-HT_{2A} since the largest concentration used (30 μM) inhibited only 49% of [^{3}H]-ketanserin binding.

This information is useful to design new neuroactive selective dopamine inhibitors based on the chemical structure of **4**.

Table 2
X-ray results

	D—H (Å)	H...A (Å)	D...A (Å)	<(D—H...A) (°)
O1—H1A...N1	0.955 (5)	1.918 (7)	2.866 (2)	172 (2)
O1—H1B...O2	0.954 (5)	1.853 (7)	2.796 (2)	169 (2)
O2—H2A...O25^{i}	0.960 (5)	1.82 (1)	2.749 (2)	161 (2)
O2—H2B...N9^{ii}	0.959 (5)	1.933 (8)	2.875 (2)	167 (2)
O25—H25...O1^{iii}	0.82	1.84	2.654 (2)	169

H-bonding geometry for compound **4**. (symmetry codes: (i) −*x*, −*y*+1, −*z*+1; (ii) *x*, *y*+1, *z*; (iii) −*x*−1, −*y*+1, −*z*+1).

Table 3
LASSBio-581 and its metabolite (**4**) affinity for D_2-*like*, 5-HT_{1A} and 5-HT_{2A} receptors

Compound	K_i (μM)[a]		
	D_2-*like*	5-HT_{1A}	5-HT_{2A}
LASSBio-581	0.95	1.2	11
Metabolite (**4**)	1.7	8.0	>19[b]

[a] K_i refers to the equilibrium dissociation constant of the compound determined in a competitive radioligand binding assay and is inversely proportional to the affinity of the compound for the receptor.
[b] Binding inhibition of 49% at 30 μM.

Acknowledgments

This work was supported by Grants from PROCAD/CAPES, Brazil (Process 0092/05-3). The authors are also grateful for the fellowships granted by CAPES.

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