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(54) Title: MICROBIAL CELL WITH IMPROVED IN VIVO CONVERSION OF THEBAINE/ORIPAVINE

(57) Abstract: A recombinant microbial host cell having improved in *vivo* conversion of reticuline and derivatives thereof (such as thebaine and/or oripavine) to relevant downstream opioids (such as neopinone, oripavine, northebaine, nororipavine or morphinone) and related compounds (such as heroin, morphine, codeine, thebaine, oripavine, oxycodone, hydrocodone, hydromorphone, oxymorphone, buprenorphine, naltrexone, naloxone or nalbuphine), wherein the microbial (such as fungal) host cell is heterologously expressing at least one functional transporter protein capable of transporting reticuline or a derivative thereof (such as thebaine and/or oripavine) and a heterologously expressed enzyme capable of acting upon reticuline or a derivative thereof. The invention also relates to uses of the microbial host cells and methods of making an opioid compound and/or opioid precursor compound and/or opioid derivative of interest.

TITLE: MICROBIAL CELL WITH IMPROVED *IN VIVO* CONVERSION OF THEBAINE/ORIPAVINE

FIELD OF THE INVENTION

The present invention relates to a recombinant microbial host cell having improved *in vivo* conversion of thebaine and/or oripavine to relevant downstream opioid related compounds, wherein the microbial host cell heterologously expresses at least one functional transporter protein. The invention also relates to use of the microbial host cell to make an opioid compound, opioid pathway intermediate, opioid derivative or opioid precursor compound of interest.

BACKGROUND ART

Opioids are alkaloid narcotics, natural or synthetic, that act on opioid receptors to produce morphine-like effects. Opioid receptors are found principally in the central and peripheral nervous system and the gastrointestinal tract. The medical uses of various opioids includes pain relief, including anesthesia, as diarrhea suppressors, as cough suppressors, and in replacement therapy for opioid use disorder.

An opiate is a narcotic drug derived from opium. Morphine, the prototypical opiate was first isolated from the flowering opium poppy plant, *Papaver somniferum*. Other examples of natural opiates also isolated from *P. somniferum*, include morphine, codeine, thebaine and oripavine.
The term opioid is a broader term for alkaloids that includes opiates and refers to any substance, natural or synthetic, that binds to the brain's opioid receptors.

Today commercially available synthetic opioid medical drug products (e.g. oxycodone, hydrocodone, hydromorphone, oxymorphone, buprenorphine, naltrexone, naloxone, nalbuphine) are obtained by chemical modification of natural opiates (e.g. thebaine) as starting precursor compounds.

For instance, the semi-synthetic opioids buprenorphine, naltrexone, naloxone and/or nalbuphine may be obtained by so-called semi-synthesis from e.g. the natural opiates thebaine or oripavine (Tomas Hudlicky; "Recent advances in process development for opiate-derived pharmaceutical agents"; Can. J. Chem. 93: 492–501 (2015)). As discussed in *Hudlicky et al.*, the natural opiates morphine and codeine are also potential starting materials for semi-synthesis of synthetic opioid medical drug products.

Figure 1 herein shows the known synthesis pathway for different opioid compounds. It illustrates that, for example, buprenorphine may be obtained by a combination of biological and chemical synthesis steps starting from natural opiates such as thebaine and/or oripavine.

Today commercially available natural and synthetic opioid medical drug products are dependent on

industrial opium poppy farming that is susceptible to environmental factors such as pests, disease, and climate, and to geopolitical factors, any of which can introduce instability and variability into this supply chain.

It is therefore desirable to establish a microbial-based manufacturing process for opioids or opioid precursors, as such a controlled, sustainable and scalable system could have the potential to address many of the current challenges associated with the opium poppy plant-based supply chain used to date.

The publication of *Galanie et al.* ("Complete biosynthesis of opioids in yeast"; Science. 2015 September 4; 349(6252): 1095–1100) describes a complete biosynthesis in engineered yeast of the opioids thebaine and hydrocodone starting from sugar.

WO2018/075670A1 describes biosynthesis in yeast of a number of the herein relevant opioids or opioid precursors, as reproduced here in Figure 1.

Dastmalchi *et al.* 2019 employs a P450 only as a reductase partner to SalSyn acting on (R)-reticuline. Indeed, Dastmalchi 2019 teaches it is preferable to engineer an alternative pathway (In90-99), rather than use a P450 because it is "a perceived bottleneck".

As known to those skilled in the art, the term "P450", also known as "cytochrome P450" or simply "CYP", refers to a broad class of over 50,000 so far identified proteins that function as monooxygenases using heme groups as co-factors tethered by a cysteine-thiolate ligand. Of particular relevance to the current invention are cytochrome P450 enzymes capable of demethylating a reticuline derivative. In some aspects, preferred reticuline derivates include thebaine and oripavine. Individual P450 enzymes in this class may be capable of N-demethylation and/or O-demethylation. Non-limiting examples of activities of P450s capable of demethylating reticuline derivatives include thebaine 6-O-demethylase, thebaine O-demethylase, thebaine N-demethylase, oripavine N-demethylase, oripavine 6-O-demethylase and/or codeine O-demethylase. In some embodiments, a P450 capable of demethylating reticuline derivatives possesses more than one of these activities. The P450s are typically membrane-associated P450s.

Figure 1 illustrates that thebaine may be converted variously into neopinone, oripavine or northebaine, and examples of suitable enzymes for doing this *in vivo* in yeast include:

- thebaine to neopinone: thebaine 6-O-demethylase (e.g. T6ODM gene) - see e.g. [00284] of WO2018/075670A1;
- thebaine to oripavine: Codeine O-demethylase (e.g. CODM gene) – see e.g. [00286] of WO2018/075670A1
- thebaine to northebaine: N-demethylase (e.g. *Bacillus* BM3 gene) – see e.g. [00303] and Figure 24 of WO2018/075670A1.

Also illustrated in Figure 1 herein, oripavine may be converted into nororipavine or morphinone, and examples of suitable enzymes for doing this *in vivo* in yeast include:

- oripavine into nororipavine: N-demethylase (e.g. *Bacillus* BM3 gene) – see e.g. [00303] and Figure 24 of WO2018/075670A1;
- oripavine into morphinone: thebaine 6-O-demethylase (e.g. T6ODM gene) - see e.g. [00284] of WO2018/075670A1.

As discussed in *Galanie et al.* the overall yield of opioids from engineered microbial-based (e.g. yeast based) manufacturing process for opioids in the art remains inadequate to the extent that such microbial-based processes are to date not the preferred options for industrial commercial production of opioids (such as e.g. buprenorphine).

As used herein, the term “membrane transport protein” (or simply “transporter”) is a membrane-bound or membrane-spanning protein involved in the movement of ions, small molecules, or macromolecules, such as peptides, across a biological membrane. A variety of transporters have evolved to move the hundreds of thousands of different substrates found in nature across suitable membranes. Of particular relevance to the current invention are transporters capable of transporting opiods and/or opioid pathway intermediates and/or opioid derivatives. An introduction to the topic can be found in Jørgensen *et al.* (“Origin and evolution of transporter substrate specificity within the NPF family”; eLife 2017;6:e19466. DOI: 10.7554/eLife.19466).

Without being limited by theory, the present inventors believe that none of the transporter proteins characterized to date are described be capable of transporting opiates like thebaine and/or oripavine into a microbial cell, such as e.g. a yeast cell.

SUMMARY OF THE INVENTION

The present invention provides an improved micorbial-based manufacturing process for the production of opioids and/or opioid precursors and/or opioid derivatives.

As discussed above and without being limited by theory, the present inventors believe that none of the transporter proteins characterized to date are described as being capable of transporting opiates like thebaine and/or oripavine into a microbial cell, such as e.g. a yeast cell.

The present inventors tested recombinant expression in *Saccharomyces cerevisiae* of a number of different transporter proteins to determine whether any of them could have a positive influence on the yield of any of several opioids. The chosen test system was the *in vivo* bioconversion of thebaine and/or oripavine to relevant downstream opioid biosynthesis compounds and intermediates.

As discussed within the working Examples herein, the inventors identified that a number of transporter proteins had no positive effect on the yields of *in vivo* bioconversions of thebaine and/or

oripavine.

However, the inventors continued their investigations and found that a number of specific transporter proteins could give a surprisingly high improvement in the yield of *in vivo* bioconversion of thebaine and/or oripavine.

For instance, as discussed in e.g. the Conclusions of Examples 4 and 5 herein, expression of one or more of the transporter genes T14_PsoNPF3_GA, T1_CjaMDR1_GA, T4_EsaGTR_GA, T7_PtrPOT_GA or T97_ScaT14_GA in a yeast strain engineered to be capable of the relevant catalysis, resulted in improved bioconversion of thebaine to northebaine in the range of 22 - 63% in comparison to the control strain without such transporters. Also, as discussed in e.g. the Conclusion of Example 6, expression of one or more of the transporter genes T65_IjaNPF_GA, T94_EcrPOT_GA and T97_ScaT14_GA resulted in improved bioconversion of thebaine to northebaine in the range of 21.8% to 31.9%. Such increases in yield are objectively a significant improvement.

The work described herein is believed to be the first time this positive “thebaine and/or oripavine improved bioconversion yield” effect has been demonstrated for a transporter protein.

There are no objective technical reasons to believe that the herein discussed membrane transporter proteins as should directly influence the *in vivo* bioconversion of thebaine and/or oripavine enzymatic reactions.

Accordingly and without being limited by theory, the improved positive bioconversion yield first demonstrated herein may be related to the herein described transporter proteins increasing the intracellular concentration of thebaine and/or oripavine (i.e. *in vivo*) in the host microbial cell.

In the working Examples described herein, the inventors noted the positive effect of improvement of the yield of *in vivo* bioconversion of thebaine and/or oripavine demonstrated for the *in vivo* conversion of thebaine into northebaine, and for oripavine into nororipavine, and for thevinone into northevinone.

However, since an objectively plausible theory of this identified positive *in vivo* conversion yield effect relates to “an increased uptake of thebaine and/or oripavine into the host cell and therefore an increased amount of thebaine and/or oripavine present *in vivo* as such” – there is no reason to believe that this positive yield effect would also not be relevant for the *in vivo* conversion of thebaine and/or oripavine into other products such as e.g. neopinone or oripavine (see Figure 1 herein).

Further, since thebaine and oripavine are structurally very similar (see e.g. Figure 1 herein) it is believed to be plausible that herein discussed membrane transporter proteins could also have a similar positive effect on oripavine as such – i.e. “increased amount of oripavine present *in vivo* as such”.

In working Examples herein, this is illustrated for e.g. T14 (se Example 4) and T97 (Example 5) that are both demonstrated to have a positive *in vivo* conversion effect for both thebaine and oripavine.

In many of the working Examples disclosed herein, the positive yield effect was demonstrated using *Saccharomyces cerevisiae* (*S. cerevisiae*) as host cell.

However, the herein discussed membrane transporter proteins are not from yeast (see table below). Many are from plants and fungi, and there is objectively no technical reason to believe that they should be optimized to only work positively in *S. cerevisiae* – to the contrary it is believed that the fact that a positive effect has herein been demonstrated for *S. cerevisiae* makes it plausible that a similar positive effect would be present for substantially all yeast host cells and many other microbial host cells.

Further, as discussed herein a number of the positively identified transporter are from fungi cells (more precisely from filamentous fungi cells). Prima facie there is no objective reason to believe that these fungi transporter should not work in a fungus cell in general – i.e. the host cell may be a fungus cell, such as e.g. a yeast cell or e.g. a filamentous fungus cell.

As discussed above, downstream to thebaine (i.e. starting from thebaine) may thebaine be converted into neopinone, oripavine or northebaine (see e.g. Figure 1 herein) and suitable enzymes for doing this *in vivo* in yeast are known in the art – for instance:
- thebaine to neopinone: thebaine 6-O-demethylase (e.g. T6ODM gene) - see e.g. [00284] of WO2018/075670A1;
- thebaine to oripavine: Codeine O-demethylase (e.g. CODM gene) – se e.g. [00286] of WO2018/075670A1
- thebaine to northebaine: N-demethylase (e.g. *Bacillus* BM3 gene) – see e.g. [00303] and Figure 24 of WO2018/075670A1.

As shown in Figure 1 herein, downstream to oripavine (i.e. starting from oripavine) may oripavine be converted into nororipavine or morphinone and suitable enzymes for doing this *in vivo* in yeast are also known in the art – for instance:
- oripavine into nororipavine: N-demethylase (e.g. *Bacillus* BM3 gene) – see e.g. [00303] and Figure 24 of WO2018/075670A1;
- oripavine into morphinone: thebaine 6-O-demethylase (e.g. T6ODM gene) - see e.g. [00284] of WO2018/075670A1.

In working examples herein, fungal N-demethylase genes/enzymes were used that are different from the bacterial N-demethylase (e.g. *Bacillus* BM3 gene) described in WO2018/075670A1. As discussed in e.g. WO2018/075670A1 and PCT/EP2018/066155 the “conversion of thebaine/oripavine” may also function in fungus host cells in general, such as e.g. a yeast cell or e.g. a filamentous

fungus cell.

The PCT/EP2018/066155 application also describes a number of different fungal O-demethylases that are suitable for the thebaine to oripavine conversion. However, PCT/EP2018/066155 does not disclose a microbial host cell, wherein the host cell expresses a P450 capable of demethylase activity on reticuline or its derivatives in combination with heterologous expression of an functional transporter protein.

In short, based on the technical disclosure herein and the prior art knowledge of the skilled person, it is routine work for the skilled person to make a recombinant fungus host cell capable of:

(1): *in vivo* conversion of thebaine into neopinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase; or

(2): *in vivo* conversion of thebaine into oripavine due to the *in vivo* presence of heterologously expressed O-demethylase; or

(3): *in vivo* conversion of thebaine into northebaine due to the *in vivo* presence of heterologously expressed N-demethylase; or

(4): *in vivo* conversion of oripavine into nororipavine due to the *in vivo* presence of heterologously expressed N-demethylase; or

(5): *in vivo* conversion of oripavine into morphinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase.

The table below provides both DNA and amino acids sequences of the positive transporter proteins discussed herein – i.e. that in working Examples herein have been recombinantly expressed in *Saccharomyces cerevisiae* and shown to have a positive influence on the yield of *in vivo* bioconversion of thebaine to relevant downstream opioid biosynthesis compounds.

Transporter gene Code	**Latin name of source organism**	**DNA SEQ ID NO:**	**Amino acid SEQ ID NO:**
T14_PsoNPF3_GA	*Papaver somniferum*	1	2
T1_CjaMDR1_GA	*Camellia japonica*	3	4
T4_EsaGTR_GA	*Eutrema salsugineum*	5	6
T7_PtrPOT_GA	*Populus trichocarpa*	7	8
T60_AmeNPF2_GA	*Argemone mexican*	9	10
T57_AcoNPF_GA	*Aquilegia coerulea*	11	12
T52_BmePTR2_GA	*Basidiobolus meristosporus*	13	14
T38_ScuPTR2_GA	*Smittium culicis*	15	16
T11_AthGTR1_GA	*Arabidopsis thaliana*	17	18
T19_RmiPTR2_GA	*Rhizopus microsporus*	19	20

T70_CmaNPF_GA	*Chelidonium majus*	21	22
T54_MelPOT_GA	*Mortierella elongate*	23	24
T65_IjaNPF_GA	*Lonicera japonica*	26	27
T94_EcrPOT_GA	*Emmonsia crescens*	28	29
T97_ScaT14_GA	*Sanguinaria canadensis*	30	31
T101_McoPUP3_1	*Macleaya cordata*	32	33
T102_PsoPUP3_1	*Papaver somniferum*	34	35
T103_PsoPUP3_2	*Papaver somniferum*	36	37
T104_PsoPUP3_3	*Papaver somniferum*	38	39
T105_PsoPUP-L	*Papaver somniferum*	40	41
T109_GflPUP3_83	*Glaucium Flavum*	42	43
T113_PsoPUP3_32	*Papaver somniferum*	44	45
T114_TorPUP3_40	*Trema orientale*	46	47
T115_CsaPUP3_48	*Cucumis sativus*	48	49
T116_HanPUP3_56	*Helianthus annuus*	50	51
T117_MacPUP3_64	*Musa acuminata subsp. malaccensis*	52	53
T121_NnuPUP3_9	*Nelumbo nucifera*	54	55
T122_PsoPUP3_17	*Papaver somniferum*	56	57
T123_PsoPUP3_25	*Papaver somniferum*	58	59
T124_PsoPUP3_33	*Papaver somniferum*	60	61
T125_JcuPUP3_41	Jatropha curcas	62	63
T126_CpePUP3_49	Cucurbita pepo subsp. pepo	64	65
T127_LsaPUP3_57	Lactuca sativa	66	67
T128_PsoPUP3_65	*Papaver somniferum*	68	69
T129_PsoPUP3_73	*Papaver somniferum*	70	71
T130_NdoPUP3_89	*Nandina domestica*	72	73
T131_PbrPUP3_81	*Papaver bracteatum*	74	75
T132_CmiPUP3_10	*Cinnamomum micranthum f. kanehirae*	76	77
T133_PsoPUP3_18	*Papaver somniferum*	78	79
T135_PsoPUP_34	*Papaver somniferum*	80	81
T136_RchPUP3_42	*Rosa chinensis*	82	83
T137_EguPUP3_50	Erythranthe guttata	84	85
T138_AduPUP3_58	Arachis duranensis	86	87
T139_PsoPUP3_66	*Papaver somniferum*	88	89
T140_PalPUP3_74	*Papaver alpinum*	90	91
T141_EcaPUP3_88	*Eschscholzia californica*	92	93

T142_McoPUP3_4	*Macleaya cordata*	94	95
T143_CmiPUP3_11	*Cinnamomum micranthum f. kanehirae*	96	97
T144_PsoPUP3_19	*Papaver somniferum*	98	99
T146_PsoPUP_35	*Papaver somniferum*	100	101
T147_MesPUP3_43	Manihot esculenta	102	103
T148_HimPUP3_51	Handroanthus impetiginosus	104	105
T149_AcoPUP3_59	*Aquilegia coerulea*	106	107
T150_PsoPUP3_67	*Papaver somniferum*	108	109
T151_PatPUP3_75	*Papaver atlanticum*	110	111
T152_GflPUP3_87	*Glaucium Flavum*	112	113
T153_PsoPUP3_5	*Papaver somniferum*	114	115
T154_CmiPUP3_12	*Cinnamomum micranthum f. kanehirae*	116	117
T156_PsoPUP3_28	*Papaver somniferum*	118	119
T157_RchPUP_36	*Rosa chinensis*	120	121
T158_DziPUP3_44	Durio zibethinus	122	123
T159_OeuPUP3_52	Olea europaea var. sylvestris	124	125
T160_CeuPUP3_60	Coffea eugenioides	126	127
T161_PsoPUP3_68	*Papaver somniferum*	128	129
T162_PmiPUP3_76	*Papaver miyabeanum*	130	131
T163_PbrPUP3_86	*Papaver bracteatum*	132	133
T164_PsoPUP3_78	*Papaver somniferum*	134	135
T165_AcoPUP3_13	*Aquilegia coerulea*	136	137
T166_PsoPUP3_21	*Papaver somniferum*	138	139
T168_FvePUP3_37	Fragaria vesca subsp. vesca	140	141
T169_ZjuPUP3_45	Ziziphus jujuba	142	143
T170_LsaPUP3_53	Lactuca sativa	144	145
T171_McoPUP3_61	*Macleaya cordata*	146	147
T172_AcoPUP3_69	*Aquilegia coerulea*	148	149
T173_PnuPUP3_77	*Papaver nudicale*	150	151
T174_PbrPUP3_85	*Papaver bracteatum*	152	153
T175_PsoPUP3_6	*Papaver somniferum*	154	155
T176_AcoPUP3_14	*Aquilegia coerulea*	155	157
T177_PsoPUP3_22	*Papaver somniferum*	158	159
T178_PsoPUP3_30	*Papaver somniferum*	160	161
T179_PyePUP3_38	Prunus yedoensis var. nudiflora	162	163
T180_McoPUP3_46	*Macleaya cordata*	164	165

T181_HanPUP3_54	*Helianthus annuus*	166	167
T182_CpaPUP3_62	Carica papaya	168	169
T184_PraPUP3_79	*Papaver radicatum*	170	171
T186_ScaPUP3_84	*Sanguinaria canadensis*	172	173
T188_AcoPUP3_15	*Aquilegia coerulea*	174	175
T189_PsoPUP3_23	*Papaver somniferum*	176	177
T191_MdoPUP3_39	Malus domestica	178	179
T192_CmiPUP3_47	*Cinnamomum micranthum f. kanehirae*	180	181
T193_AanPUP3_55	Artemisia annua	182	183
T194_CchPUP3_63	*Capsicum chinense*	184	185
T195_JcuPUP3_71	*Jatropha curcas*	186	187
T196_PtrPUP3_80	*Papaver trinifolium*	188	189

With reference to gene nomenclature, the “Transporter gene Code” may be seen as an internal code (used in e.g. Examples herein) and in the table below example sequences are connected to public known transporter protein information.

Transporter gene Code	**Official entry code**	**Database**	**Database description**
T14_PsoNPF3_GA			Relevant sequence identity to “T11_AthGTR1_GA”
T1_CjaMDR1_GA	Q94IH6_COPJA	UniProt	ABC transporter
T4_EsaGTR_GA	V4NHT1_EUTSA	UniProt	Transmembrane transporter
T7_PtrPOT_GA	B9I314_POPTR	UniProt	Transmembrane transporter activity
T60_AmeNPF2_GA		PhytoMetaSyn	Relevant sequence identity to “T11_AthGTR1_GA”
T57_AcoNPF_GA	AQUCO_00200462v1	UniProt	Transmembrane transporter activity
T52_BmePTR2_GA	K493DRAFT_340722	UniProt	Transmembrane transporter activity
T38_ScuPTR2_GA	AYI70_g3621	UniProt	Transmembrane transporter activity
T11_AthGTR1_GA	NPF2.10	UniProt	Proton-dependent glucosin-olate-specific transporter
T19_RmiPTR2_GA	BCV71DRAFT_38134	UniProt	Transmembrane transporter activity
T70_CmaNPF_GA		PhytoMetaSyn	Relevant sequence identity to

			"T11_AthGTR1_GA"
T54_MelPOT_GA	K457DRAFT_66042	UniProt	Transmembrane transporter activity
T65_IjaNPF_GA		PhytoMetaSyn	Relevant sequence identity to "T11_AthGTR1_GA"
T94_EcrPOT_GA	GX50_01424	UniProt	Transmembrane transporter activity
T97_ScaT14_GA		PhytoMetaSyn	Relevant sequence identity to "T14_PsoNPF3_GA"

As indicated in the table, the fact that e.g. "T11_AthGTR1_GA" is known to be a transporter protein can be verified by use of the "NPF2.10" entry code in the public known UniProt (https://www.uniprot.org) database.

For instance, "T14_PsoNPF3_GA" does not have an official (e.g. UniProt) entry code, since it was identified by the present inventors to be a transporter due to e.g. relevant sequence identity to "T11_AthGTR1_GA" and herein presented experimental work.

Accordingly, a first aspect of the invention relates to a recombinant microbial host cell capable of:

(a): *in vivo* conversion of thebaine into a thebaine derivative due to the *in vivo* presence of heterologously expressed enzyme; or
(b): *in vivo* conversion of oripavine into an oripavine derivative due to the *in vivo* presence of heterologously expressed enzyme; or
(c): *in vivo* conversion of thevinone into a thevinone derivative due to the *in vivo* presence of heterologously expressed enzyme.

and wherein the host cell is heterologously expressing at least one functional transporter protein capable of transporting reticuline or its derivatives.

As understood by the skilled person in the present context, the term "functional" within the term "functional transporter protein" of the first aspect simply requires that the transporter protein is capable of functioning as a transporter protein within the host cell. In contrast, as known in the art, a protein of interest may be nonfunctional due to e.g. a frameshift mutation or e.g. the insertion of a stop codon, misfolding or immediate degradation in an inappropriate host cell, or for other reasons.

As understood by the skilled person in the present context – the term "thebaine derivative" relates to a compound that thebaine may be converted into, examples of which include but are not limited to neopinone, oripavine and/or northebaine.

Similarly, the term "oripavine derivative" relates to a compound that oripavine ma converted into, examples of which include but are not limited to nororipavine and/or morphinone.

As discussed herein, an embodiment of the first aspect relates to a recombinant microbial (such as a fungus) host cell capable of:

(1): *in vivo* conversion of thebaine into neopinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase; or
(2): *in vivo* conversion of thebaine into oripavine due to the *in vivo* presence of heterologously expressed O-demethylase; or
(3): *in vivo* conversion of thebaine into northebaine due to the *in vivo* presence of heterologously expressed N-demethylase; or
(4): *in vivo* conversion of oripavine into nororipavine due to the *in vivo* presence of heterologously expressed N-demethylase; or
(5): *in vivo* conversion of oripavine into morphinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase; or

and wherein the host cell is heterologously expressing at least one functional transporter protein selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 584 of SEQ ID NO:2 (T14_PsoNPF3_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 1289 of SEQ ID NO:4 (T1_CjaMDR1_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 654 of SEQ ID NO:6 (T4_EsaGTR_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 607 of SEQ ID NO:8 (T7_PtrPOT_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 583 of SEQ ID NO:10 (T60_AmeNPF2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 594 of SEQ ID NO:12 (T57_AcoNPF_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 527 of SEQ ID NO:14 (T52_BmePTR2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with amino acids 1 to 512 of SEQ ID NO:16 (T38_ScuPTR2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 636 of SEQ ID NO:18 (T11_AthGTR1_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 565 of SEQ ID NO:20 (T19_RmiPTR2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 598 of SEQ ID NO:22 (T70_CmaNPF_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 515 of SEQ ID NO:24 (T54_MeIPOT_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 593 of SEQ ID NO:27 (T65_IjaNPF_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 604 of SEQ ID NO:29 (T94_EcrPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 604 of SEQ ID NO:31 (T97_ScaT14_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:33 (T101_McoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:35 (T102_PsoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:37 (T103_PsoPUP3_2); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:39 (T104_PsoPUP3_3); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:41 (T105_PsoPUP-L); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:43 (T109_GflPUP3_83); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:45 (T113_PsoPUP3_32); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:47 (T114_TorPUP3_40); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:49 (T115_CsaPUP3_48); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:51 (T116_HanPUP3_56); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:53 (T117_MacPUP3_64); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:55 (T121_NnuPUP3_9); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:57 (T122_PsoPUP3_17); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:59 (T123_PsoPUP3_25); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:61 (T124_PsoPUP3_33); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:63 (T125_JcuPUP3_41); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:65 (T126_CpePUP3_49); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:67 (T127_LsaPUP3_57); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:69 (T128_PsoPUP3_65); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at

least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:71 (T129_PsoPUP3_73); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:73 (T130_NdoPUP3_89); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:75 (T131_PbrPUP3_81); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:77 (T132_CmiPUP3_10); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:79 (T133_PsoPUP3_18); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:81 (T135_PsoPUP_34); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:83 (T136_RchPUP3_42); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:85 (T137_EguPUP3_50); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:87 (T138_AduPUP3_58); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:89 (T139_PsoPUP3_66); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:91 (T140_PalPUP3_74); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:93 (T141_EcaPUP3_88); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:95 (T142_McoPUP3_4); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:97 (T143_CmiPUP3_11); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:99 (T144_PsoPUP3_19); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:101 (T146_PsoPUP_35); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:103 (T147_MesPUP3_43); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:105 (T148_HimPUP3_51); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:107 (T149_AcoPUP3_59); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:109 (T150_PsoPUP3_67); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:111 (T151_PatPUP3_75); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:113 (T152_GflPUP3_87); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:115 (T153_PsoPUP3_5); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:117 (T154_CmiPUP3_12); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:119 (T156_PsoPUP3_28);

and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:121 (T157_RchPUP_36); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:123 (T158_DziPUP3_44); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:125 (T159_OeuPUP3_52); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:127 (T160_CeuPUP3_60); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:129 (T161_PsoPUP3_68); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:131 (T162_PmiPUP3_76); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:133 (T163_PbrPUP3_86); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:135 (T164_PsoPUP3_78); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:137 (T165_AcoPUP3_13); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:139 (T166_PsoPUP3_21); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:141 (T168_FvePUP3_37); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:143 (T169_ZjuPUP3_45); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:145 (T170_LsaPUP3_53); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:147 (T171_McoPUP3_61); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:149 (T172_AcoPUP3_69); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:151 (T173_PnuPUP3_77); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:153 (T174_PbrPUP3_85); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:155 (T175_PsoPUP3_6); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:157 (T176_AcoPUP3_14); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:159 (T177_PsoPUP3_22); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:161 (T178_PsoPUP3_30); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:163 (T179_PyePUP3_38); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:165 (T180_McoPUP3_46); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:167 (T181_HanPUP3_54); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:169 (T182_CpaPUP3_62); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:171 (T184_PraPUP3_79); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:173 (T186_ScaPUP3_84); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:175 (T188_AcoPUP3_15); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:177 (T189_PsoPUP3_23); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:179 (T191_MdoPUP3_39); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:181 (T192_CmiPUP3_47); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:183 (T193_AanPUP3_55); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:185 (T194_CchPUP3_63); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:187 (T195_JcuPUP3_71); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:189 (T196_PtrPUP3_80).

A second aspect of the invention relates to a method of *in vivo* producing a thebaine derivative or an oripavine derivative in a cell culture, comprising culturing the host cell of the first aspect and/or herein relevant embodiments thereof in the cell culture, under conditions;

(A): in which at least one heterologously expressed enzyme capable of the "conversion of thebaine/oripavine" of item (i) of the first aspect and/or herein relevant embodiments thereof is expressed in the host cell; and

(B): in which thebaine and/or oripavine is present *in vivo* in the host cell; and

(C): wherein the thebaine or oripavine of item (B) *in vivo* is converted into a thebaine derivative or an oripavine derivative due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production a thebaine derivative or an oripavine derivative.

A third aspect of the invention relates to a method of producing an opioid compound of interest, comprising first performing *in vivo* production of a thebaine derivative or an oripavine derivative (such as e.g. neopinone, oripavine, northebaine, nororipavine or morphinone) according to the second aspect and/or herein relevant embodiments thereof, followed by suitable *in vivo* and/or *in vitro* synthesis steps on the resulting thebaine derivative or oripavine derivative, in order to obtain the opioid compound of interest.

Embodiments of the present invention are described below by way of examples only.

DEFINITIONS

All definitions of herein relevant terms are in accordance of what would be understood by the skilled person in relation to the herein relevant technical context.

As used herein, the term “opioid pathway” refers to the multi-step synthesis of opioids and/or their derivatives. The natural synthesis of morphine is performed by a series of sequential enzymatic reactions in the opium poppy. At each step in the pathway, the product of the previous (“upstream”) reaction becomes a substrate for the next reaction. However, alternative opioid pathways can be created by substituting different enzymes to carry out a specific catalysis, or by replacing several reactions in the pathway with an alternative multi-step route to achieve the same end product opioid or opioid derivative. Since each reaction product in the pathway soon used as a substrate for the next reaction, all reaction products are known as pathway intermediates until the final opioid or opioid derivative is achieved.

As used herein, the term “opioid transporter” refers to a membrane-bound or membrane-spanning protein involved in the movement across host cell membranes of opioids and/or opioid pathway intermediates and/or opioid derivatives.

An introduction to the NPF family of transporters can be found in Jørgensen *et al*. (“Origin and evolution of transporter substrate specificity within the NPF family”; eLife 2017;6:e19466. DOI: 10.7554/eLife.19466).

As used herein, the term “reticuline or a derivative thereof” refers to precursors and intermediates in the production of opioids and opioid derivatives. In some aspects, preferred reticuline derivates of particular relevance to the transporters and enzyme activities disclosed herein include thebaine and/or oripavine.

The term "endogenous" gene refers to a gene that originates from and is produced or synthesized within a particular organism, tissue, or cell and is expressed in the same species, organism, tissue or cell for use in the technologies described herein. Therefore an endogenously expressed gene has the source organism as the host organism.

The term "heterologous" relates to a protein that is genetically engineered (such as through recombinant DNA technologies) into a cell that does not normally make (i.e., express) that protein. Therefore a heterologously expressed gene is present in a host organism that is different from the source organism for that gene.

The term “*in vitro*” (Latin: in glass) relates to studies that are conducted using components of an organism that have been isolated from their usual biological surroundings. Colloquially, these experiments are commonly called "test tube experiments". In contrast, *in vivo* studies are those that are conducted using living organisms in their normal intact state.

The term “*in vivo*” (Latin for "within the living") relates to experimentation using a whole living

organism, as opposed to a partial or dead organism, or an *in vitro* ("within the glass", e.g., in a test tube) controlled environment.

The term “biosynthetic” refers to a means of producing a compound wherein at least one step in the production process for synthesizing the compound is carried out in a recombinant biological host. In some circumstances, preferably the entire synthesis of the desired molecule is carried out in a recombinant host i.e. the entire biosynthetic pathway is present and functional within the recombinant host. In other circumstances, part of the biosynthetic pathway may be present in one host, and another part of the biosynthetic pathway may be present in another host.

The term “biotransformation” refers to the addition of a substrate to isolated cells, such that at least one enzyme endogenously or heterologously expressed in the cells are able to catalyze at least one transformation from said substrate into at least one desired product or biosynthetic pathway intermediate.

The term “recombinant host cell” is a commonly used term in the art. Within the field of genetic engineering, recombinant polynucleotide (e.g. DNA) molecules are polynucleotide (e.g. DNA) molecules that may be formed by methods of genetic recombination (such as molecular cloning) to bring together genetic material from two or more sources, creating DNA sequences that are not naturally found in biological organisms.

The term “Sequence Identity” relates to the relatedness between two amino acid sequences or between two nucleotide sequences.

For purposes of the present invention, the degree of sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled “longest identity” (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment).

For purposes of the present invention, the degree of sequence identity between two nucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled “longest identity” (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment).

DRAWINGS

Figure 1: Shows prior art known synthesis pathway for different opioid compounds

DETAILED DESCRIPTION OF THE INVENTION

A microbial host cell

As broadly known in the art, a microbe is a microscopic organism capable of existing in a single-celled form or in a colony of cells. Typically, microbes are capable of rapidly dividing into a relatively homogenous population and may be cultured by those skilled in the art very effectively under relatively simple conditions to quickly produce high densities of cells. Microbial host cells are such microbes suitable for industrial application which may be engineered (e.g. using recombinant DNA technologies) to produce one or more products of interest (such as opioids, their intermediates or derivatives). Suitable microbial host cells may be eukaryotic or prokaryotic cells. Non-limiting examples of suitable eukaryotes for scalable production of opioids, their intermediates or derivatives, include fungi such as a filamentous fungus cell or a yeast cell. Non-limiting examples of suitable prokaryotes for scalable production of opioids, their intermediates or derivatives, include bacteria, such as *E. coli, Pseudomonas sp.* or *Bacillus subtilis*. Non-limiting examples of suitable yeast cells for scalable production of opioids, their intermediates or derivatives, include

As broadly known in the art, a fungus host cell may e.g. be a yeast cell or e.g. a filamentous fungus cell.

In some circumstances, the fungus host cell is preferably a yeast cell.

The fungal host cell may e.g. be a filamentous fungus cell – such as e.g. an *Aspergillus sp.* cell, *Penicillium sp.* cell, *Trichoderma sp.* cell, *Talaromyces sp.* cell, *Asteromyces sp.* cell or *Neurospora sp.* cell.
A preferred filamentous fungus cell is an *Aspergillus sp.* cell.

For example, suitable filamentous fungus cell species can be *Aspergillus nidulans*, *Aspergillus sydowii*, *Aspergillus terreus*, *Aspergillus oryzae*, *Aspergillus caelatus*, *Aspergillus chevalieri*, *Aspergillus longivesica*, *Aspergillus parvulus*, *Aspergillus amylovorus*, *Aspergillus niger*, *Aspergillus aculeatus*, *Aspergillus ellipticus*, *Aspergillus violaceofuscus*, *Aspergillus brunneoviolaceus*, *Aspergillus japonicus*, *Aspergillus brasiliensis*, *Aspergillus aculeatinus*, *Aspergillus thermomutatus*, *Aspergillus*

implicatus, *Aspergillus acristatus*, *Penicillium bilaiae*, *Penicillium rubens*, *Penicillium chrysogenum*, *Penicillium expansum*, *Penicillium antarcticum*, *Trichoderma reesei*, *Talaromyces atroroseus*, *Asteromyces cruciatus*, or *Neurospora crassa*.

The yeast cell may e.g. be *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Candida glabrata*, *Ashbya gossypii*, *Cyberlindnera jadinii*, *Pichia pastoris*, *Kluyveromyces lactis*, *Hansenula polymorpha*, *Candida boidinii*, *Arxula adeninivorans*, *Xanthophyllomyces dendrorhous*, *Candida albicans*, *Rhodotorula sp.*, or *Rhodospiridium sp.*

In some circumstances, the yeast cell is preferably a *Saccharomycete,* most preferably, a *Saccharomyces cerevisiae* cell.

The heterologously expressed enzyme capable of the "conversion of thebaine/oripavine" of item (i) of the first aspect

Preferably, the enzyme capable of converting thebaine/oripavine" into derivatives thereof and/or further intermediates in a pathway for opioid production, is a demethylase.

As discussed above, based on the technical disclosure herein and the prior art knowledge of the skilled person, it is routine work for the skilled person to make a recombinant microbial host (e.g. yeast) capable of:

(1): *in vivo* conversion of thebaine into neopinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase; or

(2): *in vivo* conversion of thebaine into oripavine due to the *in vivo* presence of heterologously expressed O-demethylase; or

(3): *in vivo* conversion of thebaine into northebaine due to the *in vivo* presence of heterologously expressed N-demethylase; or

(4): *in vivo* conversion of oripavine into nororipavine due to the *in vivo* presence of heterologously expressed N-demethylase; or

(5): *in vivo* conversion of oripavine into morphinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase.

The skilled person knows from the prior art and/or the technical disclosure herein different suitable examples of an "enzyme capable of converting thebaine/oripavine" into derivatives thereof and/or further intermediates in a pathway for opioid production, which may be heterologously expressed as an enzyme of item (i) of the first aspect such as e.g. the ones explicitly discussed herein.

As discussed above and without being limited by theory, the improved positive yield effect demonstrated herein is probably related to a speculated ability of the herein relevant transporter proteins to transport more thebaine and/or oripavine into the host cell, thereby increasing the intracellular amount of thebaine and/or oripavine (i.e. *in vivo*) in the host cell.

Consequently, one may obtain the benefit (i.e. improved yield of derivatives "enzyme capable of converting thebaine/oripavine" into derivatives thereof and/or further intermediates in a pathway for opioid production, thereof and/or further intermediates in a pathway for opioid production) of the present invention independently of the specific "enzyme capable of converting thebaine/oripavine/thevinone" into, heterologously enzyme used – i.e. one may in principle use any suitable (e.g. prior art known) "conversion of thebaine/oripavine" heterologously enzyme of interest – i.e. this element of the present invention may be seen as an element based on prior art known knowledge for the skilled person.

Examples of suitable "conversion of thebaine/oripavine" heterologously enzyme may e.g. be:
- thebaine to neopinone: thebaine 6-O-demethylase encoded by the T6ODM gene - see e.g. [00284] of WO2018/075670A1;
- thebaine to oripavine: Codeine O-demethylase encoded by the CODM gene – se e.g. [00286] of WO2018/075670A1
- thebaine to northebaine: N-demethylase encoded by the *Bacillus* BM3 gene – see e.g. [00303] and Figure 24 of WO2018/075670A1.
- oripavine into nororipavine: N-demethylase encoded by the *Bacillus* BM3 gene – see e.g. [00303] and Figure 24 of WO2018/075670A1;
- oripavine into morphinone: thebaine 6-O-demethylase encoded by the T6ODM gene - see e.g. [00284] of WO2018/075670A1.

In working examples herein were used fungal N-demethylase genes/enzymes that are different from the bacterial N-demethylase (e.g. *Bacillus* BM3 gene) described in WO2018/075670A1.
The fungal N-demethylase based genes/enzymes used in working Example herein are described in international PCT patent application with number PCT/EP2018/066155, which was filed 18 June 2018 and not published at the filing/priority date of the present application.
The PCT/EP2018/066155 application also describes a number of different fungal O-demethylases that are suitable for the thebaine to oripavine conversion.

Accordingly, in a preferred embodiment the N-demethylase is a N-demethylase selected from the group consisting of:

- a N-demethylase comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 522 of SEQ ID NO:25 (CYPDN8).

Preferably, the recombinant fungus host cell is capable of:

(1): *in vivo* conversion of thebaine into neopinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase; or

(2): *in vivo* conversion of thebaine into oripavine due to the *in vivo* presence of heterologously expressed O-demethylase; or

(3): *in vivo* conversion of thebaine into northebaine due to the *in vivo* presence of heterologously expressed N-demethylase.

Most preferably, the recombinant fungus host cell is capable of:

(3): *in vivo* conversion of thebaine into northebaine due to the *in vivo* presence of heterologously expressed N-demethylase.

It may also be preferred that the recombinant fungus host cell is capable of:

(3): *in vivo* conversion of thebaine into northebaine due to the *in vivo* presence of heterologously expressed N-demethylase; or

(4): *in vivo* conversion of oripavine into nororipavine due to the *in vivo* presence of heterologously expressed N-demethylase.

<u>Heterologously expressing at least one functional transporter protein capable of transporting reticuline and/or its derivatives - first aspect</u>

It some embodiments it may be preferred that the transporter protein capable of transporting reticuline and/or its derivatives is a transporter protein belonging to the NRT1/PTR (NPF) transporter protein family.

The skilled person may routinely determine whether or not a transporter protein capable of transporting reticuline and/or its derivatives is an NPF transporter protein or not.

The two articles:

- Jørgensen et al. ("Origin and evolution of transporter substrate specificity within the NPF fami-ly"; eLife 2017;6:e19466. DOI: 10.7554/eLife.19466);
- Jørgensen et al. ("A Functional EXXEK Motif is Essential for Proton Coupling and Active Glucosinolate Transport by NPF2.11"; Plant Cell Physiol. 56(12): 2340–2350 (2015))

in detail describe the NPF transporter protein family and based on the definition/description of this NPF family in these articles may the skilled person routinely determine whether or not a transporter protein of interest is an NPF transporter protein or not.

As discussed in the dated 2015 article of Jørgensen – the Functional EXXEK Motif is essential for NPF – i.e. in accordance with the art, an NPF transporter protein is a protein comprising this EXXEK Motif.

It some embodiments it may be preferred that the transporter protein capable of transporting reticuline and/or its derivatives is a transporter protein belonging to the Purine Uptake Permease (PUP) transporter protein family. The PUP transporters are believed to be a distinct group of a superfamily of drug and metabolite transporters that evolved in terrestrial plant species. Jelesko J.G. 2012 ("An

expanding role for purine uptake permease-like transporters in plant secondary metabolism", Front Pnat Sci 2012;3:78. As used herein, the term "capable of PUP activity" refers to purine nucleoside transmembrane transporter activity. As used herein, the term PUP transporters refers to uptake transporters capable of enhancing *in-vivo* concentration of purine nucleobase substrates in the host, and with particular reference to the specific reactions exemplified herein, to increase the uptake of reticuline derivatives, most preferably of thebaine and/or oripavine.

As discussed above, the recombinant host cell of an embodiment of the first aspect is a microbial host cell (such as a yeast cell), wherein the microbial host cell is heterologously expressing at least one functional transporter protein capable of transporting reticuline and/or its derivatives selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 584 of SEQ ID NO:2 (T14_PsoNPF3_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 1289 of SEQ ID NO:4 (T1_CjaMDR1_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 654 of SEQ ID NO:6 (T4_EsaGTR_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 607 of SEQ ID NO:8 (T7_PtrPOT_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 583 of SEQ ID NO:10 (T60_AmeNPF2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 594 of SEQ ID NO:12 (T57_AcoNPF_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 527 of SEQ ID NO:14 (T52_BmePTR2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 512 of SEQ ID NO:16 (T38_ScuPTR2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 636 of SEQ ID NO:18 (T11_AthGTR1_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with amino acids 1 to 565 of SEQ ID NO:20 (T19_RmiPTR2_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 598 of SEQ ID NO:22 (T70_CmaNPF_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 515 of SEQ ID NO:24 (T54_MelPOT_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 593 of SEQ ID NO:27 (T65_IjaNPF_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 604 of SEQ ID NO:29 (T94_EcrPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 604 of SEQ ID NO:31 (T97_ScaT14_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:33 (T101_McoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:35 (T102_PsoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:37 (T103_PsoPUP3_2); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:39 (T104_PsoPUP3_3); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:41 (T105_PsoPUP-L); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:43 (T109_GflPUP3_83); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:45 (T113_PsoPUP3_32); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:47 (T114_TorPUP3_40); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:49 (T115_CsaPUP3_48); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:51 (T116_HanPUP3_56); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:53 (T117_MacPUP3_64); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:55 (T121_NnuPUP3_9); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:57 (T122_PsoPUP3_17); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:59 (T123_PsoPUP3_25); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:61 (T124_PsoPUP3_33); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:63 (T125_JcuPUP3_41); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:65 (T126_CpePUP3_49); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:67 (T127_LsaPUP3_57); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:69 (T128_PsoPUP3_65); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:71 (T129_PsoPUP3_73); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:73 (T130_NdoPUP3_89); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at

least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:75 (T131_PbrPUP3_81); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:77 (T132_CmiPUP3_10); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:79 (T133_PsoPUP3_18); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:81 (T135_PsoPUP_34); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:83 (T136_RchPUP3_42); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:85 (T137_EguPUP3_50); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:87 (T138_AduPUP3_58); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:89 (T139_PsoPUP3_66); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:91 (T140_PalPUP3_74); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:93 (T141_EcaPUP3_88); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:95 (T142_McoPUP3_4); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:97 (T143_CmiPUP3_11); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:99 (T144_PsoPUP3_19); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:101 (T146_PsoPUP_35); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:103 (T147_MesPUP3_43); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:105 (T148_HimPUP3_51); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:107 (T149_AcoPUP3_59); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:109 (T150_PsoPUP3_67); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:111 (T151_PatPUP3_75); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:113 (T152_GflPUP3_87); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:115 (T153_PsoPUP3_5); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:117 (T154_CmiPUP3_12); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:119 (T156_PsoPUP3_28); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:121 (T157_RchPUP_36); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:123 (T158_DziPUP3_44); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:125 (T159_OeuPUP3_52); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:127 (T160_CeuPUP3_60); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:129 (T161_PsoPUP3_68); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:131 (T162_PmiPUP3_76); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:133 (T163_PbrPUP3_86); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:135 (T164_PsoPUP3_78); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:137 (T165_AcoPUP3_13); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:139 (T166_PsoPUP3_21); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:141 (T168_FvePUP3_37); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:143 (T169_ZjuPUP3_45); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:145 (T170_LsaPUP3_53); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:147 (T171_McoPUP3_61); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:149 (T172_AcoPUP3_69); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:151 (T173_PnuPUP3_77); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:153 (T174_PbrPUP3_85); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:155 (T175_PsoPUP3_6); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:157 (T176_AcoPUP3_14); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:159 (T177_PsoPUP3_22); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:161 (T178_PsoPUP3_30); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:163 (T179_PyePUP3_38); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:165 (T180_McoPUP3_46); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:167 (T181_HanPUP3_54); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:169 (T182_CpaPUP3_62); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:171 (T184_PraPUP3_79); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:173 (T186_ScaPUP3_84); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:175 (T188_AcoPUP3_15); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:177 (T189_PsoPUP3_23); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:179 (T191_MdoPUP3_39); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:181 (T192_CmiPUP3_47); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:183 (T193_AanPUP3_55); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:185 (T194_CchPUP3_63); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:187 (T195_JcuPUP3_71); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:189 (T196_PtrPUP3_80).

It may be preferred that the host cell is a microbial host cell, wherein the microbial host cell is heterologously expressing at least two functional transporter proteins of the first aspect – for instance in working Example 5 the inventors discuss an example of a host cell that is heterologously expressing the six different functional transporter proteins SEQ ID NO:2 (T14_PsoNPF3_GA); SEQ ID NO:4 (T1_CjaMDR1_GA); SEQ ID NO:10 (T60_AmeNPF2_GA); SEQ ID NO:14 (T52_BmePTR2_GA); SEQ ID NO:18 (T11_AthGTR1_GA); SEQ ID NO:22 (T70_CmaNPF_GA).

As discussed in for example the Conclusions of Examples 4 and 5 herein, expression of one of the transporter genes T14_PsoNPF3_GA, T1_CjaMDR1_GA, T4_EsaGTR_GA, T7_PtrPOT_GA or T97_ScaT14_GA in combination with a P450 capable of demethylating reticuline and/or its derivatives in a yeast strain was shown to improve bioconversion of thebaine to northebaine in the range of 22 - 63% in comparison to the control strain. This is objectively a significant improvement.

Accordingly, preferably the recombinant host cell of the first aspect is a microbial host cell, wherein the microbial host cell (such as a yeast host cell) is heterologously expressing a P450 capable of demethylating reticuline and/or its derivatives and also heterologously expressing at least one functional transporter protein capable of transporting reticuline and/or its derivatives selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 584 of SEQ ID NO:2 (T14_PsoNPF3_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 1289 of SEQ ID NO:4 (T1_CjaMDR1_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 654 of SEQ ID NO:6 (T4_EsaGTR_GA);
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at

least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 607 of SEQ ID NO:8 (T7_PtrPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 604 of SEQ ID NO:31 (T97_ScaT14_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:33 (T101_McoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:35 (T102_PsoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:37 (T103_PsoPUP3_2); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:39 (T104_PsoPUP3_3); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:41 (T105_PsoPUP-L); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:43 (T109_GflPUP3_83); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:45 (T113_PsoPUP3_32); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:47 (T114_TorPUP3_40); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:49 (T115_CsaPUP3_48); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:51 (T116_HanPUP3_56); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:53 (T117_MacPUP3_64); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:55 (T121_NnuPUP3_9); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:57 (T122_PsoPUP3_17); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:59 (T123_PsoPUP3_25); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:61 (T124_PsoPUP3_33); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:63 (T125_JcuPUP3_41); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:65 (T126_CpePUP3_49); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:67 (T127_LsaPUP3_57); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:69 (T128_PsoPUP3_65); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:71 (T129_PsoPUP3_73); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:73 (T130_NdoPUP3_89); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:75 (T131_PbrPUP3_81); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:77 (T132_CmiPUP3_10); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:79 (T133_PsoPUP3_18); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:81 (T135_PsoPUP_34); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:83 (T136_RchPUP3_42); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:85 (T137_EguPUP3_50); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:87 (T138_AduPUP3_58); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:89 (T139_PsoPUP3_66); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:91 (T140_PalPUP3_74); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:93 (T141_EcaPUP3_88); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:95 (T142_McoPUP3_4); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:97 (T143_CmiPUP3_11); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:99 (T144_PsoPUP3_19); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:101 (T146_PsoPUP_35); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:103 (T147_MesPUP3_43); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:105 (T148_HimPUP3_51); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:107 (T149_AcoPUP3_59); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:109 (T150_PsoPUP3_67); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:111 (T151_PatPUP3_75); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:113 (T152_GflPUP3_87); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:115 (T153_PsoPUP3_5); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:117 (T154_CmiPUP3_12); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:119 (T156_PsoPUP3_28); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:121 (T157_RchPUP_36); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:123 (T158_DziPUP3_44); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:125 (T159_OeuPUP3_52); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:127 (T160_CeuPUP3_60); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at

least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:129 (T161_PsoPUP3_68); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:131 (T162_PmiPUP3_76); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:133 (T163_PbrPUP3_86); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:135 (T164_PsoPUP3_78); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:137 (T165_AcoPUP3_13); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:139 (T166_PsoPUP3_21); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:141 (T168_FvePUP3_37); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:143 (T169_ZjuPUP3_45); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:145 (T170_LsaPUP3_53); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:147 (T171_McoPUP3_61); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably

at least 99%) identity with the amino acid sequence of SEQ ID NO:149 (T172_AcoPUP3_69); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:151 (T173_PnuPUP3_77); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:153 (T174_PbrPUP3_85); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:155 (T175_PsoPUP3_6); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:157 (T176_AcoPUP3_14); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:159 (T177_PsoPUP3_22); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:161 (T178_PsoPUP3_30); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:163 (T179_PyePUP3_38); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:165 (T180_McoPUP3_46); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:167 (T181_HanPUP3_54); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:169 (T182_CpaPUP3_62);

and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:171 (T184_PraPUP3_79); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:173 (T186_ScaPUP3_84); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:175 (T188_AcoPUP3_15); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:177 (T189_PsoPUP3_23); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:179 (T191_MdoPUP3_39); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:181 (T192_CmiPUP3_47); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:183 (T193_AanPUP3_55); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:185 (T194_CchPUP3_63); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:187 (T195_JcuPUP3_71); and

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with the amino acid sequence of SEQ ID NO:189 (T196_PtrPUP3_80).

As discussed above and in working examples 4 and 5 herein, "T14_PsoNPF3_GA" and T97_ScaT14_GA are demonstrated to have a positive *in vivo* conversion effect for both thebaine and oripavine.

Accordingly, in some embodiments, the recombinant microbial host cell of the first aspect is a microbial cell, wherein the microbial host cell (such as yeast host cell) is heterologously expressing at least one functional transporter protein selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 584 of SEQ ID NO:2 (T14_PsoNPF3_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 99%) identity with amino acids 1 to 604 of SEQ ID NO:31 (T97_ScaT14_GA).

<u>A method of *in vivo* producing a thebaine derivative or an oripavine derivative (such as e.g. neopinone, oripavine, northebaine, nororipavine or morphinone) – second aspect</u>

As discussed above, a second aspect of the invention relates to a method of *in vivo* producing a thebaine derivative or an oripavine derivative in a cell culture, comprising culturing the microbial host cell of the first aspect and/or herein relevant embodiments thereof in the cell culture, under conditions;

(A): in which at least one of the heterologously expressed enzyme capable of "conversion of thebaine and/or oripavine" of the first aspect herein relevant embodiments thereof is thereby present *in vivo* in the host cell; and

(B): in which thebaine and/or oripavine is present *in vivo* in the host cell; and

(C): wherein the thebaine or oripavine of item (B) *in vivo* is converted into a thebaine derivative or an oripavine derivative due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production a thebaine derivative or an oripavine derivative.

A preferred embodiment of the second aspect relates to a method of *in vivo* producing neopinone, oripavine, northebaine, nororipavine or morphinone in a cell culture, comprising culturing the host cell of the first aspect and/or herein relevant embodiments thereof in the cell culture, under conditions;

(A): in which at least one of the "conversion of thebaine/oripavine" heterologously enzyme of item (1 - 5) of an embodiment of the first aspect and/or herein relevant embodiments thereof is expressed and thereby present *in vivo* in the host cell; and

(B): in which thebaine and/or oripavine is present *in vivo* in the host cell; and

(C): wherein the thebaine and/or oripavine of item (B) *in vivo* is converted into neopinone, oripavine, northebaine, nororipavine or morphinone due to the presence of the heterologously expressed

enzyme of item (A) in order to thereby get *in vivo* production of neopinone, oripavine, northebaine, nororipavine or morphinone.

In relation to item (B) - thebaine and/or oripavine may be present *in vivo* in the host cell via e.g.:
- thebaine and/or oripavine *in vivo* biosynthesis within the host cell; and/or
- thebaine and/or oripavine may be present in a cell culture medium or reaction medium and then taken up by the host cell in order for the thebaine and/or oripavine to be present *in vivo* in the host cell for biotransformation.

As discussed herein - thebaine and/or oripavine *in vivo* biosynthesis within a microbial host cell (such as a yeast host cell) is well known in the art.
It is also well known (see e.g. working examples herein) how to prepare thebaine and/or oripavine supplemented cell culture medium or reaction medium to be taken up by the yeast host cell in order for the thebaine and/or oripavine to be present *in vivo* in the microbial host cell.

As discussed above, the improved positive yield effect demonstrated herein is probably related to that the herein relevant transporter proteins increase the intracellular amount of thebaine and/or oripavine (i.e. *in vivo*) in the fungus host cell because more thebaine and/or oripavine is transported into the host yeast cell. This advantageous effect is also relevant in relation to *in vivo* biosynthesis within the host cell of thebaine/oripavine, since some of the thebaine/oripavine may be exported out of the host cell and herein relevant transporter proteins can then transport the thebaine/oripavine back into the host cell again.

Using methods known in the art, the in item (C) produced neopinone, oripavine, northebaine, nororipavine or morphinone may be isolated in order to get a substantially pure (e.g. at least 20%, 30%, 50%, 60% or at least 90% pure w/w) composition of the compound(s). Alternatively, they may e.g. *in vivo* be converted to further herein relevant downstream compounds (see e.g. Figure 1 herein and the Third Aspect, below).

In short, based on the technical disclosure herein and the prior art knowledge of the skilled person – it is routine work for the skilled person to perform the method of the second aspect and/or herein relevant embodiments thereof.

In relation to the second aspect – it may be preferred that it is a method for producing neopinone, oripavine or northebaine, wherein

(A): at least one of the "conversion of thebaine" heterologously enzyme of item (1), (2) or (3) of the first aspect and/or herein relevant embodiments thereof is expressed and thereby present *in vivo* in the host cell; and

(B): in which thebaine is present *in vivo* in the host cell; and

(C): wherein the thebaine of item (B) *in vivo* is converted into neopinone, oripavine or northebaine

due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production of neopinone, oripavine or northebaine.

In relation to the second aspect – it may be preferred that it is a method for producing oripavine or northebaine, wherein

(A): at least one of the "conversion of thebaine" heterologously enzyme of item (2) or (3) of the first aspect and/or herein relevant embodiments thereof is expressed and thereby present *in vivo* in the host cell; and

(B): in which thebaine is present *in vivo* in the host cell; and

(C): wherein the thebaine of item (B) *in vivo* is converted into oripavine or northebaine due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production of oripavine or northebaine.

Most preferably is a method for producing northebaine, wherein

(A): at least one of the "conversion of thebaine" heterologously enzyme of item (3) of the first aspect and/or herein relevant embodiments thereof is expressed and thereby present *in vivo* in the host cell; and

(B): in which thebaine is present *in vivo* in the host cell; and

(C): wherein the thebaine of item (B) *in vivo* is converted into northebaine due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production of northebaine.

Further, and in relation to the second aspect, it may be preferred that it is a method for producing oripavine, northebaine or nororipavine, wherein

(A): at least one of the "conversion of thebaine" heterologously enzyme of item (2), (3) or (4) of the first aspect and/or herein relevant embodiments thereof is expressed and thereby present *in vivo* in the host cell; and

(B): in which thebaine and/or oripavine is present *in vivo* in the host cell; and

(C): wherein the thebaine of item (B) *in vivo* is converted into oripavine or northebaine or the oripavine of item (B) *in vivo* is converted into nororipavine due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production of oripavine, northebaine or nororipavine.

Preferably, the method of the second aspect and/or herein relevant embodiments thereof is a method, wherein there in item (C) of the second aspect is an increased *in vivo* conversion of thebaine and/or oripavine due to that the cultured host cell is heterologously expressing at least one functional transporter protein (e.g. T14_PsoNPF3_GA) of the first aspect and/or herein relevant embodiments thereof; and

wherein the "increased *in vivo* conversion of thebaine and/or oripavine" is understood to be relative to an otherwise identical performed method using an otherwise identical control host cell that is not

heterologously expressing at least one functional transporter protein (e.g. T14_PsoNPF3_GA) of the first aspect and/or herein relevant embodiments thereof.

The "increased *in vivo* conversion of thebaine and/or oripavine" is understood to be relative to an otherwise identical control host cell, which is not heterologously expressing at least one functional transporter protein (e.g. T14_PsoNPF3_GA) of the first aspect.
The skilled person knows or can easily identify (by e.g. routine genome sequencing) an "otherwise identical control host cell" with no heterologously expressing of at least one functional transporter protein (e.g. T14_PsoNPF3_GA) of the first aspect.
If for instance the yeast host cell is heterologously expressing e.g. T14_PsoNPF3_GA – then is the method of the second aspect and/or herein relevant embodiments thereof simply performed with the host cell heterologously expressing T14_PsoNPF3_GA and the otherwise identical control host cell with no expressing of T14_PsoNPF3_GA and the amount of *in vivo* conversion of thebaine and/or oripavine is then measured (e.g. by LC-MS) - if the use of the host cell with expressing of T14_PsoNPF3_GA is giving increased *in vivo* conversion as compared to the control host cell then it is understood that there is an increased *in vivo* conversion of thebaine and/or oripavine due to that the host cell is heterologously expressing T14_PsoNPF3_GA.

A method of producing an opioid compound of interest - Third Aspect

As discussed above, a third aspect of the invention relates to a method of producing an opioid compound of interest, comprising first performing *in vivo* production of a thebaine derivative or an oripavine derivative (such as e.g. neopinone, oripavine, northebaine, nororipavine or morphinone) according to the second aspect and/or herein relevant embodiments thereof followed by suitable *in vivo* and/or *in vitro* synthesis steps in order to obtain the opioid compound of interest.

A preferred embodiment of the third aspect relates to a method of producing an opioid compound of interest, comprising first performing *in vivo* production of neopinone, oripavine, northebaine, nororipavine or morphinone according to the second aspect and/or herein relevant embodiments thereof followed by suitable *in vivo* and/or *in vitro* synthesis steps in order to obtain the opioid compound of interest.

In short, based on the technical disclosure herein and the prior art knowledge of the skilled person – it is routine work for the skilled person to perform the method of the third aspect and/or herein relevant embodiments thereof.
As discussed herein, starting from neopinone, oripavine, northebaine, nororipavine or morphinone - suitable *in vivo* and/or *in vitro* synthesis steps in order to obtain the opioid compound of interest are well known in the art. See for example WO2018/211331 and Sipos *et al.* (2009).
As understood – the term "*in vitro* synthesis steps" may e.g. relate to suitable chemical synthesis steps as e.g. illustrated for buprenorphine in Figure 1 herein.

Preferably, the opioid compound of interest is heroin, morphine, codeine, thebaine, oripavine, oxycodone, hydrocodone, hydromorphone, oxymorphone, buprenorphine, naltrexone, naloxone, nalmefene, noroxymorphone or nalbuphine. In some embodiments, most preferably the opioid compound of interest is buprenorphine, nalmefene or noroxymorphone.

EXAMPLES

As discussed above, the amino acid sequence for P450 CYPDN8 N-demethylase from *Rhizopus microspores* is shown as SEQ ID NO. 9 herein and discussed in international PCT patent application with number PCT/EP2018/066155, which was filed 18 June 2018.

PCT patent application with number PCT/EP2018/066155 also describes other herein relevant technical details such as e.g. further details in relation to herein referred pOD75 and pOD13 plasmids.

Accordingly, based on the technical disclosure herein and the technical disclosure of PCT patent application with number PCT/EP2018/066155 – the skilled person can routinely carry out the relevant technical matter of the present invention – such as e.g. the relevant working Examples herein.

Example 1: Strain Engineering

Saccharomyces cerevisiae yeast strains were constructed in strain background EVST25898 (genotype MATalpha his3Δ0 leu2Δ0 ura3Δ0 aro3Δ::pTEF1-ARO4(K229L)-tCYC1::pPGK1-ARO7(T266L)-tADH1::KI CAT5-91Met GAL2 ho MIP1-661Thr SAL1-1 YORWΔ22::npBIO1nt20npBIO6nt).

The EVST25898 with the genotype above corresponds to S288C (genotype MATalpha his3Δ0 leu2Δ0 ura3Δ0). S288C is a publicly available widely used laboratory strain (see the Saccharomyces Genome Database (SGD)). As is known from other works, one would get similar results by use of EVST25898 with genotype above or by use of S288C (genotype MATalpha his3Δ0 leu2Δ0 ura3Δ0) as background/control strains, since these two host phenotypes are substantially identical.

Plasmid based gene expression

Strain was transformed with relevant plasmids using the lithium acetate method (Gietz *et al.* 2002. Methods Enzymol. Vol 350, p87-96).

For testing the impact of possible transporter proteins on the bioconversion of Thebaine to Northebaine, the host yeast strain was transformed with a plasmid containing cytochrome P450 gene CYPDN8 N-demethylase from *Rhizopus microspores* (pOD75) along with a plasmid containing Cel_CPR (co) from *Cunninghamella elegans* (pOD13) in combination with the various possible transporter proteins. Genes were inserted and expressed using either P413TEF, P415TEF or p416TEF, all described by *Mumberg et al.*, 1995. Gene. Apr 14;156(1):119-22.

The control strain was constructed by transforming strain EVST25898 with pOD75, pOD13 as well as an empty plasmid: p416TEF.

Table 1 describes the plasmids that were expressed with the yeast strains. Transformants were selected in synthetic complete (SC) agar plates lacking histidine, leucine and uracil. Transformation plates were incubated for 3-4 days at 30°C until visible colonies were obtained.

Table 1: Plasmids expressed in the corresponding yeast strains

Vector name	**Backbone**	**Promoter-Gene-Terminator**	**Yeast Selection Marker**	**Description**
pOD13	P413TEF	pTEF1-Cel_CPR_co-tCYC1	HIS3	Cel_CPR (co) from *Cunninghamella elegans*
pOD75	P415TEF	pTEF1-CYPDN8-tCYC1	LEU2	A0A0C7AZL4 (co) from *Rhizopus microsporus*
p416TEF		No gene inserted	URA3	Mumberg et al., 1995. Gene. Apr 14;156(1):119-22

Gene expression by integration.

Strain EVST25898 was modified by genomic integration using the *Saccharomyces cerevisiae* gene integration and expression system developed by *Mikkelsen, MD* et al. (Metab. Eng. 14, Issue 2, 104-111 (2012)). The cytochrome P450 gene CYPDN8, N-demethylase from *Rhizopus microspores* was expressed using the well-known *Saccharomyces cerevisiae* TEF1 promoter, and the Cel_CPR (co) from *Cunninghamella elegans* was expressed using the *Saccharomyces cerevisiae* PGK1 promoter. The expression cassette was integrated in site XII-5 using the *Kluyveromyces lactis URA3* marker as selection marker for growth on media lacking uracil (described by *Mikkelsen, MD et al*. (Metab. Eng. 14, Issue 2, 104-111 (2012)). Subsequently, the transporter genes T11_AthGTR1_GA (SEQ ID NO: 17), T52_BmePTR2_GA (SEQ ID NO: 13), T14_PsoNPF3_GA (SEQ ID NO: 1), T60_AmeNPF2_GA (SEQ ID NO: 9), T1_CjaMDR1_GA (SEQ ID NO: 3) and T70_CmaNPF_GA (SEQ ID NO: 21) were integrated into the site XI-5 of the *Saccharomyces cerevisiae* strain using the *Saccharomyces cerevisiae* TEF1, PGK1, TEF2, TDH3, TPI1, and PDC1 promoters respectively. Selection for transformants was done using the well-known *Kluyveromyces lactis LEU2* marker available e.g. from EUROSCARF (http://www.euroscarf.de) and growth on media lacking leucine. After that, plasmid pOD13 (see Table 1) was transformed with the resulting strain in order to make the strain prototrophic by selecting on media lacking histidine. Transformation plates were incubated for 3-4 days at 30°C until visible colonies were obtained.

Example 2. Cultivation and harvest of yeast strains

Cultivation. Yeast strains were cultivated in 96-deep-well-plate (DWP) format. Cells were grown in 0.5 ml SC-His-Leu-Ura medium at 30°C with shaking at 250 rpm in ISF1-X Kuhner shaker for 20-24 hours and utilized as precultures for *in vivo* bioconversion assays.

50 µl of the overnight cell cultures were grown in 450 µl Synthetic complete (SC)-His-Leu-Ura medium (pH 7) or DELFT minimal medium (pH 7) containing 0.5 mM thebaine or oripavine. Both media contain 0.1 M potassium phosphate buffer.

Thebaine (or Oripavine) were added via a 25 mM stock solution in DMSO. Cells were grown for 72 hours with shaking at 250 rpm.

Harvest. 50 µl of cell cultures were transferred to a new 96-deep-well-plate containing 50 µl of MilliQ water. The harvested 96 well plate was incubated at 80°C for 10 minutes. Plate was then centrifugated for 10 minutes at 4000 rpm. The supernatants were then diluted in MilliQ water to reach a final dilution of 1:100. Thebaine, northebaine, oripavine and nororipavine contents were analyzed by LC-MS.

Example 3. LC-MS procedures

For all compounds (Thebaine, Northebaine, Oripavine and Nororipavine) stock solutions were prepared in DMSO at a concentration of 10mM. Standard solutions were prepared at concentrations of 6µM, 4µM, 2µM, 1µM, 500nM, 200nM, 100nM, 50nM, 20nM and 10nM from the stock solutions. Samples were injected into the Agilent 1290 UPLC coupled to an Ultivo Triple Quadrupole. The LC-MS method was as follows: Mobile Phase A. H_2O + 0.1% Formic acid; Mobile Phase B: Acetonitrile + 0.1% Formic acid; Column: Phenomenex Kinetex 1.7µm XB-C18 100Å, 2.1x100mm. The elution gradient is shown in Table 2 and the LC-MS conditions are given in Table 3. Table 4 shows the mass spectrometer source and detector parameters and Table 5 shows the target compounds, their retention times, their parent ion, transition ions (MRM) as well as dwell times, cone voltages and collision energies used.

Table 2: Gradient for LC-MS

Time (min)	% B
0	2
0.30	2
4.00	30
4.40	100
4.90	100
5	2
6	2

Table 3: LC-MS conditions

Parameter	Value

Injection volume	2 µl
Column Temperature	30°C ± 4°C
Injection method	Flow through needle
Flow	0.4 ml/min
Auto sampler temperature	10°C ± 2°C
Reconditioning wash	2% Acetonitrile (in H_2O), 5 sec
Weak wash	20% Methanol (in H_2O), 5 sec
Strong wash	30% Acetonitrile, 30% Methanol, 30% 2-Propanol, 10% H_2O, 10 sec
Seal wash	20% 2-Propanol (in H_2O)

Table 4: Mass spectrometer source and detector parameters (Ultivo Triple Quadrupole)

Source Parameter	**Value**
Ion Source	Electrospray Positive Mode (ESI+)
Capillary Voltage	3.5 kV
Nozzle Voltage	500 V
Source Gas Temperature	290°C
Source Gas Flow	12 L/min
Source Sheath Gas Temperature	380°C
Source Sheath Gas Flow	12 L/min
Nebulizer	30 psi
Mode	MS/MS
Collision	See Table 4

Table 5. Multiple reaction monitoring targets and conditions (ESI +)

Target compound	**Retention time (min)**	**Parent ion (m/z)**	**Daughter ion (m/z)**	**Dwell time (ms)**	**Fragmentor voltage (V)**	**Collision energy (V)**
Northebaine	3.53	298	249	55.03	100	20
Thebaine	3.6	312	58	61.53	110	10
Oripavine	2.59	298	237	64.05	110	5
Nororipavine	2.54	284	218	70.30	110	10

Example 4. Identification of transporters capable of improving bioconversion of thebaine and/or derivatives thereof

Bioconversion. Expression of transporter genes in a strain containing cytochrome P450 gene CYPDN8 and cytochrome P450 reductase Cel_CPR (co) gave remarkable improvement in bioconversion of thebaine to northebaine for some of the transporter genes, where some exhibited a significant improved bioconversion when strains were grown in presence of 0.5mM thebaine.

Table 6: Bioconversion of thebaine to northebaine in strains expressing different possible transporter enzymes and improvement in the bioconversion as compared to control strain not expressing any heterologous transporter genes.

Transporter genes	Percentage bioconversion of Thebaine to Northebaine (%)	Improvement in Thebaine to Northebaine bioconversion (%)	Growth medium
T1_CjaMDR1_GA	12.0	45	SC-his-leu-ura
T3_NcaNPF_GA	6.7	-19	SC-his-leu-ura
T4_EsaGTR_GA	11.3	36	SC-his-leu-ura
T5_AlyPOT_GA	6.1	-27	SC-his-leu-ura
T6_CruGTR_GA	6.4	-23	SC-his-leu-ura
T7_PtrPOT_GA	13.5	63	SC-his-leu-ura
T8_BnaMFS_GA	4.2	-49	SC-his-leu-ura
T10_BolGTR_GA	6.0	-28	SC-his-leu-ura
T11_AthGTR1_GA	9.7	17	SC-his-leu-ura
T12_PsoNPF1_GA	6.7	-19	SC-his-leu-ura
T14_PsoNPF3_GA	10.3	24	SC-his-leu-ura
T17_PsoNPF6_GA	5.1	-39	SC-his-leu-ura
Control SC-his-leu-ura	8.3	0.0	SC-his-leu-ura
T18_PsoNPF7_GA	*10.0*	*2*	DELFT minimal medium
T19_RmiPTR2_GA	9.5	13	DELFT minimal medium
T20_RmiPTR2_v2_GA	7.9	-6	DELFT minimal medium
T21_RalPTR2_GA	7.3	-13	DELFT minimal medium
T22_CanPOT_GA	4.4	-48	DELFT minimal medium
T23_ArePOT_GA	4.6	-45	DELFT minimal medium
T24_SlyPTR2_GA	4.0	-52	DELFT minimal medium
T25_AorPOT_GA	4.1	-51	DELFT minimal medium
T26_NfuPOT_GA	4.0	-52	DELFT minimal medium
T28_MciPOT_GA	4.2	-50	DELFT minimal medium
T29_AcaPOT_GA	5.1	-39	DELFT minimal medium
T30_MlyPOT_GA	5.6	-33	DELFT minimal medium
T31_TgaPOT_GA	4.4	-48	DELFT minimal medium
T32_AarPOT_GA	5.1	-39	DELFT minimal medium
T33_CcuPTR2_GA	4.6	-45	DELFT minimal medium
T34_HvePOT_GA	5.5	-35	DELFT minimal medium
T35_EcuPOT_GA	7.8	-7	DELFT minimal medium
T36_RnePOT_GA	4.3	-49	DELFT minimal medium
T37_OcoPOT_GA	4.8	-45	DELFT minimal medium

T38_ScuPTR2_GA	9.9	18	DELFT minimal medium
T39_CgrPOT_GA	5.6	-33	DELFT minimal medium
T40_EdePOT_GA	6.1	-27	DELFT minimal medium
T41_CalPTR2_GA	5.7	-32	DELFT minimal medium
T44_CcaMFS_GA	4.4	-48	DELFT minimal medium
T45_PanPOT_GA	*9.8*	*0*	DELFT minimal medium
T46_RchPOT_GA	8.1	-4	DELFT minimal medium
T47_PbeNPF_GA	4.6	-45	DELFT minimal medium
T48_CcaPOT_GA	*9.7*	*-1*	DELFT minimal medium
T49_HanPOT_GA	7.7	-8	DELFT minimal medium
T51_TorPOT_GA	5.5	-35	DELFT minimal medium
T52_BmePTR2_GA	*11.7*	*19*	DELFT minimal medium
T53_EhePOT_GA	7.3	-13	DELFT minimal medium
T54_MelPOT_GA	*10.9*	*11*	DELFT minimal medium
T55_NsyNPF_GA	3.2	-62	DELFT minimal medium
T56_CanNPF_GA	8.4	0	DELFT minimal medium
T57_AcoNPF_GA	*11.7*	*19*	DELFT minimal medium
T59_AmeNPF1_GA	5.3	-37	DELFT minimal medium
T60_AmeNPF2_GA	*11.9*	*21*	DELFT minimal medium
T61_TwiNPF_GA	8.1	-4	DELFT minimal medium
T62_SmaNPF_GA	7.5	-11	DELFT minimal medium
T63_CfoNPF_GA	7.4	-12	DELFT minimal medium
T64_XsiNPF_GA	6.9	-18	DELFT minimal medium
T66_TelNPF_GA	8.3	-1	DELFT minimal medium
T69_PhoNPF_GA	5.4	-36	DELFT minimal medium
T70_CmaNPF_GA	9.1	8	DELFT minimal medium
T72_TcoNPF_GA	8.4	0	DELFT minimal medium
T73_PbrNPF1_GA	5.8	-31	DELFT minimal medium
T74_PbrNPF2_GA	6.6	-21	DELFT minimal medium
T75_PbrNPF3_GA	7.7	-8	DELFT minimal medium
T76_AhuNPF_GA	4.9	-42	DELFT minimal medium
T77_PocNPF_GA	5.5	-35	DELFT minimal medium
T78_VofNPF_GA	8.5	1	DELFT minimal medium
T79_EcaNPF_GA	7.6	-10	DELFT minimal medium
T80_CroNPF_GA	*9.8*	*0*	DELFT minimal medium
T82_NsaNPF_GA	*8.8*	*-10*	DELFT minimal medium
Control DELFT	8.4	0.0	DELFT minimal medium
Control DELFT	*9.8*	*0.0*	*DELFT minimal medium*

Numbers in *Italic* are relative to Control DELFT of 9.8.

Improvement of bioconversion:

Expression of one of the transporter genes T14_PsoNPF3_GA, T1_CjaMDR1_GA, T4_EsaGTR_GA or T7_PtrPOT_GA in a yeast strain that contains cytochrome P450 gene CYPDN8 and cytochrome

P450 reductase Cel_CPR (co), results in improved bioconversion of thebaine to northebaine in the range of 24 - 63% in comparison to the control strain.
Further, significant improvement was also seen for the transporter genes T60_AmeNPF2_GA, T57_AcoNPF_GA, T52_BmePTR2_GA, T38_ScuPTR2_GA, T11_AthGTR1_GA, T19_RmiPTR2_GA, T70_CmaNPF_GA or T54_MelPOT_GA.

Conclusions

The results of this Example demonstrate expression of one of the transporter genes T14_PsoNPF3_GA, T1_CjaMDR1_GA, T4_EsaGTR_GA or T7_PtrPOT_GA in a yeast strain that contains cytochrome P450 gene CYPDN8 and cytochrome P450 reductase Cel_CPR (co), results in improved bioconversion of thebaine to northebaine in the range of 24 - 63% in comparison to the control strain.
Further, significant improvement was also seen for the transporter genes T60_AmeNPF2_GA, T57_AcoNPF_GA, T52_BmePTR2_GA, T38_ScuPTR2_GA, T11_AthGTR1_GA, T19_RmiPTR2_GA, T70_CmaNPF_GA or T54_MelPOT_GA.

Further, transporters were tested for improvement in conversion of the thebaine derivative Oripavine to Nororipavine

Bioconversion. Expression of transporter gene T14_PsoNPF3_GA from *Papaver somniferum* in a strain containing cytochrome P450 gene CYPDN8 and cytochrome P450 reductase Cel_CPR (co) showed remarkable improvement in bioconversion of oripavine to nororipavine. In an assay where a strain was grown in presence of 0.5mM oripavine, the strain containing T14_PsoNPF3_GA exhibited 2.3% bioconversion of the oripavine to nororipavine, which corresponds to an improvement in bioconversion of oripavine to nororipavine by 64% in comparison to the control strain.

Table 7: Bioconversion and improvement in oripavine to nororipavine bioconversion compared to the control strain, observed when growing strains expressing different possible transporter proteins.

Transporter genes	Bioconversion of oripavine to nororipavine (%)	Improvement of oripavine to nororipavine bioconversion as compared to control (%)
T14_PsoNPF3_GA	2.3	64
Control	1.4	0

Conclusions

The result of this Example demonstrate that expression of transporter gene T14_PsoNPF3_GA gave around 64% more bioconversion of oripavine to nororipavine – which is a remarkable yield improvement.

Example 5. Identification of further transporters capable of improving bioconversion of thebaine and/or derivatives thereof

This Example 5 discusses transporter genes that are not explicitly mentioned in corresponding Example 4 above.

Bioconversion. In bioconversion experiments similar to Example 4 above – 3 additional transporters have shown to improve bioconversion of thebaine to northebaine.
As shown in Table 8 below, T65_IjaNPF_GA, T94_EcrPOT_GA and T97_ScaT14_GA are able to improve bioconversion of thebaine to northebaine by 29.9%, 31.9% and 21.8%, respectively, when compared to a control strain.

Table 8 also shows a yeast strain which genes CYPDN8 from *Rhizopus microspores* and Cel_CPR_co from *Cunninghamella elegans* have been integrated into host strain EVST25898 (Example 1) at Chromosome XII-5 with URA3 from *Kluyveromyces lactis* as selection marker. Subsequently, 6 different transporters T11_AthGTR1_GA, T52_BmePTR2_GA, T14_PsoNPF3_GA, T60_AmeNPF2_GA, T1_CjaMDR1_GA, and T70_CmaNPF_GA were expressed in the same strain at Chromosome XI-5 with LEU2 from *Kluyveromyces lactis* as selection marker. Plasmid pOD13 (Table 1) was also expressed in the same strain to make the strain prototrophic. An indication of improvement in the bioconversion of thebaine to northebaine when multiple copies of various transporters were expressed in the same strain.

Table 8: Bioconversion of thebaine to northebaine in strains expressing different possible transporter enzymes and improvement in the bioconversion as compared to control strain not expressing any heterologous transporter genes.

Transporter genes	Percentage bioconversion of Thebaine to Northebaine (%)	Improvement in Thebaine to Northebaine bioconversion (%)	Growth medium
T65_IjaNPF_GA	10.9	29.9	DELFT minimal medium
T94_EcrPOT_GA	11.1	31.9	DELFT minimal medium
T97_ScaT14_GA	10.2	21.8	DELFT minimal medium
T11_AthGTR1_GA + T52_BmePTR2_GA + T14_PsoNPF3_GA +	11.3	34.6	DELFT minimal medium

T60_AmeNPF2_GA + T1_CjaMDR1_GA + T70_CmaNPF_GA			
Control DELFT	8.4		DELFT minimal medium

When multiple of different genes were expressed in the yeast cell, it is referred to as gene1 + gene2, etc.

Conclusions

In bioconversion experiments similar to Example 4 above – the results of this Example demonstrate that three additional transporters have shown to improve bioconversion of thebaine to northebaine. As shown in Table 8, T65_IjaNPF_GA, T94_EcrPOT_GA and T97_ScaT14_GA are able to improve bioconversion of thebaine to northebaine by 29.9%, 31.9% and 21.8%, respectively, when compared to a control strain.

Further, a strain comprising a combination of 6 transporter proteins discussed in Example 4 gave a very good improvement of thebaine to northebaine.

Further, transporters were tested for improvement in conversion of the thebaine derivative Oripavine to Nororipavine

Bioconversion. In bioconversion experiments similar to Example 4 above – an additional transporter that is able to help improving bioconversion of oripavine to nororipavine has been identified.
As shown in Table 9 below, T97_ScaT14_GA from *Sanguinaria canadensis* is able to convert close to 5% of oripavine to nororipavine when fed with 0.5mM oripavine. In comparison to the control strain, expression of T97_ScaT14_GA improves the bioconversion of oripavine to nororipavine by 254.4%.

Table 9: Bioconversion and improvement in oripavine to nororipavine bioconversion compared to the control strain.

Transporter genes	**Bioconversion of oripavine to nororipavine (%)**	**Improvement of oripavine to nororipavine bioconversion as compared to control (%)**
T97_ScaT14_GA	4.96	254.4
Control	1.4	0

Conclusions

In bioconversion experiments similar to Example 4 above, the results of this Example demonstrate an additional transporter able to help in improving bioconversion of oripavine to nororipavine has been identified.

As shown in Table 9, T97_ScaT14_GA from *Sanguinaria canadensis* is able to convert close to 5% of oripavine to nororipavine when fed with 0.5mM oripavine. In comparison to the control strain, expression of T97_ScaT14_GA improves the bioconversion of oripavine to nororipavine by 254.4%.

Example 6. Identification of Purine Uptake Permease (PUP) transporters capable of improving bioconversion of thebaine

Bioconversion. The impact of purine uptake permease transporter proteins on bioconversion of thebaine to northebaine was studied by transforming yeast strain with a plasmid containing a cytochrome P450 comparable to the above examples that was capable of acting on reticuline derivatives such as thebaine and/or oripavine using the backbone plasmid p415TEF. A plasmid containing cytochrome P450 reductase (pOD13 from Example 1) was also expressed in combination with various candidate transporter proteins. Yeast strain construction and method of screening for PUP transporters were as previously described in Example 1. Table 10 shows the result of percentage bioconversion from thebaine to northebaine with the expression of various PUP transporters. Table 10 also presents the percentage improvement in the bioconversion when normalized for a control strain expressing P450 but not expressing any heterologous transporter.

Table 10: Percentage P450-mediated bioconversion from Thebaine to Northebaine with the expression of various transporters and percentage improvements in the bioconversion as compared to a control strains not expressing any heterologous transporters.

PUP Transporters	Percentage bioconversion of Thebaine to Northebaine (%)	Percentage Improvement in Thebaine to Northebaine bioconversion (%)
T101_McoPUP3_1	7.0	6.7
T102_PsoPUP3_1	8.6	29.8
T103_PsoPUP3_2	7.1	7.9
T104_PsoPUP3_3	7.4	11.4
T105_PsoPUP-L	9.2	39.8
Control 1	6.6	-
T109_GflPUP3_83	6.4	55.0
T122_PsoPUP3_17	6.1	48.4
T130_NdoPUP3_89	4.9	19.9
T131_PbrPUP3_81	4.9	20.4
T132_CmiPUP3_10	6.6	60.2
T133_PsoPUP3_18	5.9	42.7
T136_RchPUP3_42	4.6	11.1
T137_EguPUP3_50	5.1	24.7
T138_AduPUP3_58	4.6	11.7
T139_PsoPUP3_66	4.9	19.5
T140_PalPUP3_74	5.4	30.5
T141_EcaPUP3_88	6.8	64.7

T142_McoPUP3_4	7.7	88.9
T143_CmiPUP3_11	5.8	41.8
T144_PsoPUP3_19	7.7	87.1
T146_PsoPUP_35	4.6	13.4
T147_MesPUP3_43	6.1	49.8
T148_HimPUP3_51	5.0	21.4
T149_AcoPUP3_59	6.9	69.1
T150_PsoPUP3_67	5.9	43.6
T151_PatPUP3_75	5.7	39.1
T152_GflPUP3_87	8.0	94.0
T153_PsoPUP3_5	4.9	19.1
T154_CmiPUP3_12	7.1	74.2
T157_RchPUP_36	5.8	42.1
T159_OeuPUP3_52	5.8	41.8
T160_CeuPUP3_60	5.4	30.9
T161_PsoPUP3_68	6.2	51.9
T162_PmiPUP3_76	6.4	56.1
T163_PbrPUP3_86	5.1	24.8
T164_PsoPUP3_78	5.2	27.2
T165_AcoPUP3_13	6.5	57.9
T166_PsoPUP3_21	6.6	61.9
T168_FvePUP3_37	6.4	56.5
T169_ZjuPUP3_45	6.6	60.6
T170_LsaPUP3_53	6.7	62.6
T171_McoPUP3_61	5.5	33.8
T172_AcoPUP3_69	6.6	60.2
T174_PbrPUP3_85	5.3	29.4
T175_PsoPUP3_6	6.7	63.9
T176_AcoPUP3_14	5.8	41.5
T177_PsoPUP3_22	6.5	57.4
T178_PsoPUP3_30	6.1	47.6
T180_McoPUP3_46	5.5	35.0
T181_HanPUP3_54	5.3	30.1
T182_CpaPUP3_62	6.9	67.8
T184_PraPUP3_79	5.2	27.9
T186_ScaPUP3_84	7.0	69.8
T188_AcoPUP3_15	4.7	14.7
T189_PsoPUP3_23	4.7	14.8
T191_MdoPUP3_39	5.2	26.5
T192_CmiPUP3_47	5.5	35.0
T193_AanPUP3_55	6.2	51.9
T194_CchPUP3_63	5.7	39.1
T195_JcuPUP3_71	5.3	29.8
T196_PtrPUP3_80	5.7	39.1

Control 2	4.1	-

Note: Control 1 is used as the control for T101_McoPUP3_1, T102_PsoPUP3_1, T103_PsoPUP3_2, T104_PsoPUP3_3 and T105_PsoPUP-L. Control 2 is used as control for the rest of the PUP transporters. This was done to compensate for any slight variations that may arise between different runs of LC-MS analysis.

Improvement of bioconversion. When compared to a control strain without a heterologous transporter, several strains engineered with PUP transporters exhibited at least 50% greater bioconversion of the 0.5mM thebaine fed in this assay. Amongst the PUP transporters examined, PUP transporters T152_GflPUP3_87, T149_AcoPUP3_59, T109_GflPUP3_83, T142_McoPUP3_4, T144_PsoPUP3_19, T141_EcaPUP3_88, T182_CpaPUP3_62, T193_AanPUP3_55 and T122_PsoPUP3_17 exhibited improvements in bioconversion of thebaine to northebaine in the range of 48 - 94% in comparison to the control strain without a heterologous transporter (Table 10). Expression of some PUP transporters, such as T152_GflPUP3_87 from *Glaucium flavum*, T149_AcoPUP3_59 from *Aquilegia coerulea*, and T142_McoPUP3_4 from *Macleaya cordata*, gave remarkable improvements in the P450-mediated bioconversion of thebaine to northebaine.

Table 11: Purine Uptake Permease transporters which have been demonstrated herein to provide especially large improvements in the P450-mediated bioconversion from Thebaine to Northebaine.

PUP Transporters	Latin Name for Origin of Sourced Genes
T152_GflPUP3_87	*Glaucium flavum*
T142_McoPUP3_4	*Macleaya cordata*
T144_PsoPUP3_19	*Papaver somniferum*
T149_AcoPUP3_59	*Aquilegia coerulea*
T109_GflPUP3_83	*Glaucium flavum*
T141_EcaPUP3_88	*Eschscholzia californica*
T182_CpaPUP3_62	*Carica papaya*
T193_AanPUP3_55	*Artemisia annua*
T132_CmiPUP3_10	*Cinnamomum micranthum f. kanehirae*
T186_ScaPUP3_84	*Sanguinaria canadensis*
T175_PsoPUP3_6	*Papaver somniferum*
T122_PsoPUP3_17	*Papaver somniferum*

Conclusions

Table 11 shows some of the PUP transporters that have been herein demonstrated for the first time to shown very considerable improvements in the bioconversion from Thebaine to Northebaine by P450s. In particular, the results of this Example demonstrate that expression of PUP transporters T152_GflPUP3_87 from *Glaucium flavum*, T149_AcoPUP3_59 from *Aquilegia coerulea*, T109_GflPUP3_83 from *Glaucium flavum*, T142_McoPUP3_4 from *Macleaya cordata*, T144_PsoPUP3_19 from *Papaver somniferum*, T141_EcaPUP3_88 from *Eschscholzia californica*, T182_CpaPUP3_62 from *Carica papaya*, T193_AanPUP3_55 from *Artemisia annua*, T132_CmiPUP3_10 from *Cinnamomum micranthum f. kanehirae*, T186_ScaPUP3_84 from

Sanguinaria canadensis, T175_PsoPUP3_6 from *Papaver somniferum* and T122_PsoPUP3_17 from *Papaver somniferum*, each stimulated somewhere in the range of 48-94% more bioconversion of thebaine to northebaine. Theimprovements in yield shown herein are both unexpected and highly valuable given the nature of the opioid-related compounds produced.

Example 7. Identification of Purine Uptake Permease (PUP) transporters capable of improving bioconversion of Oripavine to Nororipavine

Bioconversion. The impact of purine uptake permease transporter proteins on bioconversion of oripavine to nororipavine was studied by transforming yeast with a plasmid containing a comparable cytochrome P450 that was capable of acting on reticuline derivatives such as thebaine and/or oripavine using the backbone plasmid p415TEF. A plasmid containing cytochrome P450 reductase (pOD13 from Example 1) was also expressed in combination with various possible transporter proteins. Yeast strain construction and method of screening for PUP transporters were as previously described in Example 1. Table 12 shows the result of percentage bioconversion from oripavine to nororipavine with the expression of various PUP transporters. Table 12 also presents the percentage improvement in the bioconversion when normalized for a control strain expressing P450 but not expressing any heterologous transporter.

Improvement of bioconversion. The percentage bioconversion of strains displayed by several PUP transporters exhibited as high as 1600% and greater bioconversion of the 0.5 mM oripavine fed to the assay when compared to a control strain expressing P450 but not expressing transporter. Amongst the transporters examined in this example, PUP transporters T149_AcoPUP3_59, T168_FvePUP3_37, T116_HanPUP3_56, T192_CmiPUP3_47, T109_GflPUP3_83, T180_McoPUP3_46, T193_AanPUP3_55, T165_AcoPUP3_13, T195_JcuPUP3_71 and T143_CmiPUP3_11 exhibited improvements in the P450-mediated bioconversion of oripavine to nororipavine in the range of 1400 - 1662% in comparison to the control strain expressing P450 but not expressing a heterologous transporter (Table 12). Expression of some PUP transporters, such as T149_AcoPUP3_59 from *Aquilegia coerulea*, T168_FvePUP3_37 from *Fragaria vesca subsp. vesca*, and T116_HanPUP3_56 from *Helianthus annuus* gave particularly remarkable improvements in the P450-mediated bioconversion of oripavine to nororipavine.

Table 12: Percentage of P450-mediated bioconversion from Oripavine to Nororipavine with the expression of various transporters and the percentage improvement in the bioconversion as compared to a control strains not expressing any heterologous transporters.

PUP Transporters	Percentage Bioconversion of Oripavine to Nororipavine (%)	Percentage Improvement in Oripavine to Nororipavine bioconversion (%)
T101_McoPUP3_1	3.5	147.7
T102_PsoPUP3_1	10.1	621.4
T103_PsoPUP3_2	1.7	21.9
T104_PsoPUP3_3	8.0	474.8

T105_PsoPUP-L	12.2	771.1
Control 1	1.4	-
T109_GflPUP3_83	15.5	1447.6
T113_PsoPUP3_32	10.1	912.6
T114_TorPUP3_40	5.9	486.0
T115_CsaPUP3_48	11.7	1065.5
T116_HanPUP3_56	17.5	1653.1
T117_MacPUP3_64	4.2	317.4
T121_NnuPUP3_9	1.5	47.1
T122_PsoPUP3_17	12.5	1149.3
T123_PsoPUP3_25	1.3	32.4
T124_PsoPUP3_33	4.9	393.9
T125_JcuPUP3_41	14.5	1346.2
T126_CpePUP3_49	11.8	1077.1
T127_LsaPUP3_57	5.4	441.7
T128_PsoPUP3_65	4.8	383.9
T129_PsoPUP3_73	6.3	532.3
T130_NdoPUP3_89	14.2	1315.0
T131_PbrPUP3_81	5.0	399.4
T132_CmiPUP3_10	14.8	1383.1
T133_PsoPUP3_18	14.5	1349.6
T135_PsoPUP_34	1.7	73.4
T136_RchPUP3_42	13.0	1197.8
T137_EguPUP3_50	8.4	744.8
T138_AduPUP3_58	14.5	1348.7
T139_PsoPUP3_66	4.4	341.0
T140_PalPUP3_74	3.6	264.7
T141_EcaPUP3_88	11.3	1030.8
T142_McoPUP3_4	15.4	1438.8
T143_CmiPUP3_11	15.8	1483.1
T144_PsoPUP3_19	15.1	1408.2
T146_PsoPUP_35	5.8	478.0
T147_MesPUP3_43	10.5	954.4
T148_HimPUP3_51	7.7	674.8
T149_AcoPUP3_59	17.4	1639.5
T150_PsoPUP3_67	13.4	1240.3
T151_PatPUP3_75	13.2	1223.8
T152_GflPUP3_87	14.9	1394.9
T153_PsoPUP3_5	6.8	583.2
T154_CmiPUP3_12	11.4	1039.5
T156_PsoPUP3_28	6.9	589.7
T157_RchPUP_36	12.2	1123.8
T158_DziPUP3_44	7.7	673.2

T159_OeuPUP3_52	10.0	902.7
T160_CeuPUP3_60	4.0	304.6
T161_PsoPUP3_68	13.4	1237.9
T162_PmiPUP3_76	14.1	1314.8
T163_PbrPUP3_86	3.8	280.2
T164_PsoPUP3_78	5.5	448.3
T165_AcoPUP3_13	15.3	1429.8
T166_PsoPUP3_21	10.3	931.0
T168_FvePUP3_37	17.6	1662.4
T169_ZjuPUP3_45	14.1	1310.9
T170_LsaPUP3_53	14.7	1372.2
T171_McoPUP3_61	3.5	251.3
T172_AcoPUP3_69	12.3	1126.4
T173_PnuPUP3_77	1.9	94.1
T174_PbrPUP3_85	5.5	452.5
T175_PsoPUP3_6	8.7	769.9
T176_AcoPUP3_14	7.4	636.2
T177_PsoPUP3_22	11.3	1029.5
T178_PsoPUP3_30	15.0	1396.5
T179_PyePUP3_38	4.4	344.5
T180_McoPUP3_46	16.8	1580.6
T181_HanPUP3_54	12.6	1160.4
T182_CpaPUP3_62	14.5	1349.5
T184_PraPUP3_79	3.3	234.2
T186_ScaPUP3_84	10.6	962.2
T188_AcoPUP3_15	3.0	197.8
T189_PsoPUP3_23	8.3	729.7
T191_MdoPUP3_39	9.5	849.2
T192_CmiPUP3_47	17.2	1618.5
T193_AanPUP3_55	15.5	1454.4
T194_CchPUP3_63	2.1	110.0
T195_JcuPUP3_71	15.1	1413.6
T196_PtrPUP3_80	10.9	986.6
Control 2	1.0	-

Note: Control 1 is used as the control for T101_McoPUP3_1, T102_PsoPUP3_1, T103_PsoPUP3_2, T104_PsoPUP3_3 and T105_PsoPUP-L. Control 2 is used as control for the rest of the PUP transporters. This is was done to account for any slight variations that may arise from different runs of LC-MS analysis.

Table 13: Purine Uptake Permease transporters which have demonstrated herein to provide especially large improvements in the P450-mediated bioconversion of Oripavine to Nororipavine.

Transporter Genes	**Latin Name for Origin of Sourced Genes**
T149_AcoPUP3_59	*Aquilegia coerulea*

T168_FvePUP3_37	*Fragaria vesca subsp. vesca*
T116_HanPUP3_56	*Helianthus annuus*
T192_CmiPUP3_47	*Cinnamomum micranthum f. kanehirae*
T109_GflPUP3_83	*Glaucium Flavum*
T180_McoPUP3_46	*Macleaya cordata*
T193_AanPUP3_55	*Artemisia annua*
T165_AcoPUP3_13	*Aquilegia coerulea*
T195_JcuPUP3_71	*Jatropha curcas*
T143_CmiPUP3_11	*Cinnamomum micranthum f. kanehirae*

Conclusions

Table 13 shows some of the PUP transporters that have been demonstrated herein for the first time to shown particularly high improvements in the P450-mediated bioconversion of oripavine to nororipavine. Amongst the transporters examined in this example, PUP transporters T149_AcoPUP3_59 from *Aquilegia coerulea*, T168_FvePUP3_37 from *Fragaria vesca subsp. vesca*, T116_HanPUP3_56 from *Helianthus annuus*, T192_CmiPUP3_47 from *Cinnamomum micranthum f. kanehirae*, T109_GflPUP3_83 from *Glaucium flavum*, T180_McoPUP3_46 from *Macleaya cordata*, T193_AanPUP3_55 from *Artemisia annua*, T165_AcoPUP3_13 from *Aquilegia coerulea*, T195_JcuPUP3_71 from *Jatropha curcas* and T143_CmiPUP3_11 from *Cinnamomum micranthum f. kanehirae*, exhibited improvements in the range of 1400-1662% more P450-mediated bioconversion of thebaine to northebaine in comparison to the control strain expressing P450 but not expressing a heterologous transporter. Such improvements in yield are particularly remarkable and represent a significant step forward towards a sustainable, secure, and scalable biosynthetic means of producing these compounds.

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CLAIMS

1. A recombinant microbial host cell capable of:

(a): *in vivo* conversion of thebaine into a thebaine derivative due to the *in vivo* presence of heterologously expressed enzyme; or
(b): *in vivo* conversion of oripavine into an oripavine derivative due to the *in vivo* presence of heterologously expressed enzyme; or
(c) *in vivo* conversion of thevinone into a thevinone derivative due to the *in vivo* presence of heterologously expressed enzyme;

and wherein the host cell is heterologously expressing at least one transporter protein capable of improving host cell uptake of a reticuline derivative for *in vivo* conversion.

2. The recombinant host cell of claim 1 expressing at least one transporter protein capable of improving host cell uptake of a reticuline derivative, wherein the reticuline derivative is at least one of thebaine, oripavine and thevinone.

3. The recombinant host cell of any preceding claim, wherein the heterologously expressed enzyme is a P450 capable of demethylase activity on thebaine and/or oripavine and/or thevinone.

4. The recombinant host cell of any preceding claim, wherein the recombinant microbial host cell is a host cell capable of:

(1): *in vivo* conversion of thebaine into neopinone due to the *in vivo* presence of heterologously expressed thebaine 6-O-demethylase; or
(2): *in vivo* conversion of thebaine into oripavine due to the *in vivo* presence of heterologously expressed O-demethylase; or
(3): *in vivo* conversion of thebaine into northebaine due to the *in vivo* presence of heterologously expressed N-demethylase; or
(4): *in vivo* conversion of oripavine into nororipavine due to the *in vivo* presence of heterologously expressed N-demethylase; or
(5): *in vivo* conversion of oripavine into morphinone due to the *in vivo* presence of heterologously expressed oripavine 6-O-demethylase.

5. The recombinant host cell of any of the preceding claims, wherein the transporter protein is a transporter protein belonging to the NRT1/PTR (NPF) transporter protein family or the purine permease (PUP) transporter family.

6. The recombinant host cell of any of the preceding claims, wherein the host cell is heterologously expressing at least one functional transporter protein selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 584 of SEQ ID NO:2 (T14_PsoNPF3_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 1289 of SEQ ID NO:4 (T1_CjaMDR1_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 654 of SEQ ID NO:6 (T4_EsaGTR_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 607 of SEQ ID NO:8 (T7_PtrPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 583 of SEQ ID NO:10 (T60_AmeNPF2_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 594 of SEQ ID NO:12 (T57_AcoNPF_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 527 of SEQ ID NO:14 (T52_BmePTR2_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 512 of SEQ ID NO:16 (T38_ScuPTR2_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 636 of SEQ ID NO:18 (T11_AthGTR1_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 565 of SEQ ID NO:20 (T19_RmiPTR2_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 598 of SEQ ID NO:22 (T70_CmaNPF_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 515 of SEQ ID NO:24 (T54_MelPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 593 of SEQ ID NO:27 (T65_IjaNPF_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 604 of SEQ ID NO:29 (T94_EcrPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 604 of SEQ ID NO:31 (T97_ScaT14_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:33 (T101_McoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:35 (T102_PsoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:37 (T103_PsoPUP3_2); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:39 (T104_PsoPUP3_3); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:41 (T105_PsoPUP-L); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:43 (T109_GflPUP3_83); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:45 (T113_PsoPUP3_32); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:47 (T114_TorPUP3_40); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:49 (T115_CsaPUP3_48); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:51 (T116_HanPUP3_56); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:53 (T117_MacPUP3_64); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:55 (T121_NnuPUP3_9); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:57 (T122_PsoPUP3_17); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:59 (T123_PsoPUP3_25); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:61 (T124_PsoPUP3_33); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:63 (T125_JcuPUP3_41); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:65 (T126_CpePUP3_49); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:67 (T127_LsaPUP3_57); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:69 (T128_PsoPUP3_65); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:71 (T129_PsoPUP3_73); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:73 (T130_NdoPUP3_89); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:75 (T131_PbrPUP3_81); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:77 (T132_CmiPUP3_10); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:79 (T133_PsoPUP3_18); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:81 (T135_PsoPUP_34); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:83 (T136_RchPUP3_42); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:85 (T137_EguPUP3_50); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:87 (T138_AduPUP3_58); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:89 (T139_PsoPUP3_66); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:91 (T140_PalPUP3_74); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:93 (T141_EcaPUP3_88); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:95 (T142_McoPUP3_4); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:97 (T143_CmiPUP3_11); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:99 (T144_PsoPUP3_19); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:101 (T146_PsoPUP_35); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:103 (T147_MesPUP3_43); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:105 (T148_HimPUP3_51); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:107 (T149_AcoPUP3_59); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:109 (T150_PsoPUP3_67); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:111 (T151_PatPUP3_75); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:113 (T152_GflPUP3_87); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:115 (T153_PsoPUP3_5); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:117 (T154_CmiPUP3_12); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:119 (T156_PsoPUP3_28); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:121 (T157_RchPUP_36); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:123 (T158_DziPUP3_44); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:125 (T159_OeuPUP3_52); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:127 (T160_CeuPUP3_60); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:129 (T161_PsoPUP3_68); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:131 (T162_PmiPUP3_76); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:133 (T163_PbrPUP3_86); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:135 (T164_PsoPUP3_78); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:137 (T165_AcoPUP3_13); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:139 (T166_PsoPUP3_21); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:141 (T168_FvePUP3_37); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:143 (T169_ZjuPUP3_45); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:145 (T170_LsaPUP3_53); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:147 (T171_McoPUP3_61); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:149 (T172_AcoPUP3_69); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:151 (T173_PnuPUP3_77); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:153 (T174_PbrPUP3_85); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:155 (T175_PsoPUP3_6); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:157 (T176_AcoPUP3_14); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:159 (T177_PsoPUP3_22); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:161 (T178_PsoPUP3_30); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:163 (T179_PyePUP3_38); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:165 (T180_McoPUP3_46); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:167 (T181_HanPUP3_54); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:169 (T182_CpaPUP3_62); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:171 (T184_PraPUP3_79); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:173 (T186_ScaPUP3_84); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:175 (T188_AcoPUP3_15); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:177 (T189_PsoPUP3_23); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:179 (T191_MdoPUP3_39); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:181 (T192_CmiPUP3_47); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:183 (T193_AanPUP3_55); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:185 (T194_CchPUP3_63); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:187 (T195_JcuPUP3_71); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:189 (T196_PtrPUP3_80).

7. The recombinant host cell of any of claims 1-6, wherein the host cell is heterologously expressing at least one functional transporter protein capable of transporting thebaine, selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 607 of SEQ ID NO:8 (T7_PtrPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 1289 of SEQ ID NO:4 (T1_CjaMDR1_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 654 of SEQ ID NO:6 (T4_EsaGTR_GA); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 583 of SEQ ID NO:10 (T60_AmeNPF2_GA); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 43 (T109_GflPUP3_83); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 57 (T122_PsoPUP3_17); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 77 (T132_CmiPUP3_10); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 79 (T133_PsoPUP3_18); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 93 (T141_EcaPUP3_88); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 95 (T142_McoPUP3_4); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 97 (T143_CmiPUP3_11); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 99 (T144_PsoPUP3_19); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 103 (T147_MesPUP3_43); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 107 (T149_AcoPUP3_59); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 109 (T150_PsoPUP3_67); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 113 (T152_GflPUP3_87); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 117 (T154_CmiPUP3_12); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 121 (T157_RchPUP_36); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 125 (T159_OeuPUP3_52); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 129 (T161_PsoPUP3_68); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with

SEQ ID NO: 131 (T162_PmiPUP3_76); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 137 (T165_AcoPUP3_13); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 139 (T166_PsoPUP3_21); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 141 (T168_FvePUP3_37); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 143 (T169_ZjuPUP3_45); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 145 (T170_LsaPUP3_53); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 149 (T172_AcoPUP3_69); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 155 (T175_PsoPUP3_6); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 157 (T176_AcoPUP3_14); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 159 (T177_PsoPUP3_22); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 161 (T178_PsoPUP3_30); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 169 (T182_CpaPUP3_62); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 173 (T186_ScaPUP3_84); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 183 (T193_AanPUP3_55).

8. The recombinant host cell of any of claims 1-6, wherein the host cell is heterologously expressing at least one functional transporter protein capable of transporting oripavine, selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 70% identity with amino acids 1 to 604 of SEQ ID NO:31 (T97_ScaT14_GA); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 43 (T109_GflPUP3_83); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with

SEQ ID NO: 45 (T113_PsoPUP3_32); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 49 (T115_CsaPUP3_48); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 51 (T116_HanPUP3_56); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 57 (T122_PsoPUP3_17); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 63 (T125_JcuPUP3_41); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 65 (T126_CpePUP3_49); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 73 (T130_NdoPUP3_89); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 77 (T132_CmiPUP3_10); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 79 (T133_PsoPUP3_18); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 83 (T136_RchPUP3_42); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 87 (T138_AduPUP3_58); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 93 (T141_EcaPUP3_88); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 95 (T142_McoPUP3_4); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 97 (T143_CmiPUP3_11); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 99 (T144_PsoPUP3_19); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 103 (T147_MesPUP3_43); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 107 (T149_AcoPUP3_59); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 109 (T150_PsoPUP3_67); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 111 (T151_PatPUP3_75); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 113 (T152_GflPUP3_87); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 117 (T154_CmiPUP3_12); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 121 (T157_RchPUP_36); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 125 (T159_OeuPUP3_52); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 129 (T161_PsoPUP3_68); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 131 (T162_PmiPUP3_76); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 137 (T165_AcoPUP3_13); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 139 (T166_PsoPUP3_21); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 141 (T168_FvePUP3_37); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 143 (T169_ZjuPUP3_45); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 145 (T170_LsaPUP3_53); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 149 (T172_AcoPUP3_69); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 155 (T175_PsoPUP3_6); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 159 (T177_PsoPUP3_22); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 161 (T178_PsoPUP3_30); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 165 (T180_McoPUP3_46); and
- a transporter protein comprising an amino acid sequence which has at least 70% identity with

SEQ ID NO: 167 (T181_HanPUP3_54); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 169 (T182_CpaPUP3_62); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 173 (T186_ScaPUP3_84); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 179 (T191_MdoPUP3_39); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 181 (T192_CmiPUP3_47); and

- a transporter protein comprising an amino acid sequence which has at least 70% identity with SEQ ID NO: 183 (T193_AanPUP3_55).

9. The recombinant host cell of any of the preceding claims, wherein the host cell is heterologously expressing at least one functional transporter protein selected from the group consisting of:

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 584 of SEQ ID NO:2 (T14_PsoNPF3_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 1289 of SEQ ID NO:4 (T1_CjaMDR1_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 654 of SEQ ID NO:6 (T4_EsaGTR_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 607 of SEQ ID NO:8 (T7_PtrPOT_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 583 of SEQ ID NO:10 (T60_AmeNPF2_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 594 of SEQ ID NO:12 (T57_AcoNPF_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 527 of SEQ ID NO:14 (T52_BmePTR2_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 512 of SEQ ID NO:16 (T38_ScuPTR2_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 636 of SEQ ID NO:18 (T11_AthGTR1_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 565 of SEQ ID NO:20 (T19_RmiPTR2_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 598 of SEQ ID NO:22 (T70_CmaNPF_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 515 of SEQ ID NO:24 (T54_MelPOT_GA);

- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 593 of SEQ ID NO:27 (T65_IjaNPF_GA);
- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 604 of SEQ ID NO:29 (T94_EcrPOT_GA); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with amino acids 1 to 604 of SEQ ID NO:31 (T97_ScaT14_GA).

10. The recombinant host cell of any of claims 1-9, wherein the host cell is heterologously expressing at least one functional transporter protein selected from the group consisting of:
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:33 (T101_McoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:35 (T102_PsoPUP3_1); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:37 (T103_PsoPUP3_2); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:39 (T104_PsoPUP3_3); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:41 (T105_PsoPUP-L); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:43 (T109_GflPUP3_83); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:45 (T113_PsoPUP3_32); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:47 (T114_TorPUP3_40); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:49 (T115_CsaPUP3_48); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:51 (T116_HanPUP3_56); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:53 (T117_MacPUP3_64); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:55 (T121_NnuPUP3_9); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:57 (T122_PsoPUP3_17); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:59 (T123_PsoPUP3_25); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:61 (T124_PsoPUP3_33); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with

the amino acid sequence of SEQ ID NO:63 (T125_JcuPUP3_41); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:65 (T126_CpePUP3_49); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:67 (T127_LsaPUP3_57); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:69 (T128_PsoPUP3_65); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:71 (T129_PsoPUP3_73); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:73 (T130_NdoPUP3_89); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:75 (T131_PbrPUP3_81); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:77 (T132_CmiPUP3_10); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:79 (T133_PsoPUP3_18); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:81 (T135_PsoPUP_34); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:83 (T136_RchPUP3_42); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:85 (T137_EguPUP3_50); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:87 (T138_AduPUP3_58); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:89 (T139_PsoPUP3_66); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:91 (T140_PalPUP3_74); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:93 (T141_EcaPUP3_88); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:95 (T142_McoPUP3_4); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:97 (T143_CmiPUP3_11); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:99 (T144_PsoPUP3_19); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:101 (T146_PsoPUP_35); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with

the amino acid sequence of SEQ ID NO:103 (T147_MesPUP3_43); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:105 (T148_HimPUP3_51); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:107 (T149_AcoPUP3_59); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:109 (T150_PsoPUP3_67); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:111 (T151_PatPUP3_75); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:113 (T152_GflPUP3_87); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:115 (T153_PsoPUP3_5); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:117 (T154_CmiPUP3_12); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:119 (T156_PsoPUP3_28); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:121 (T157_RchPUP_36); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:123 (T158_DziPUP3_44); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:125 (T159_OeuPUP3_52); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:127 (T160_CeuPUP3_60); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:129 (T161_PsoPUP3_68); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:131 (T162_PmiPUP3_76); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:133 (T163_PbrPUP3_86); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:135 (T164_PsoPUP3_78); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:137 (T165_AcoPUP3_13); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:139 (T166_PsoPUP3_21); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:141 (T168_FvePUP3_37); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with

the amino acid sequence of SEQ ID NO:143 (T169_ZjuPUP3_45); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:145 (T170_LsaPUP3_53); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:147 (T171_McoPUP3_61); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:149 (T172_AcoPUP3_69); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:151 (T173_PnuPUP3_77); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:153 (T174_PbrPUP3_85); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:155 (T175_PsoPUP3_6); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:157 (T176_AcoPUP3_14); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:159 (T177_PsoPUP3_22); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:161 (T178_PsoPUP3_30); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:163 (T179_PyePUP3_38); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:165 (T180_McoPUP3_46); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:167 (T181_HanPUP3_54); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:169 (T182_CpaPUP3_62); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:171 (T184_PraPUP3_79); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:173 (T186_ScaPUP3_84); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:175 (T188_AcoPUP3_15); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:177 (T189_PsoPUP3_23); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:179 (T191_MdoPUP3_39); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:181 (T192_CmiPUP3_47); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with

the amino acid sequence of SEQ ID NO:183 (T193_AanPUP3_55); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:185 (T194_CchPUP3_63); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:187 (T195_JcuPUP3_71); and
- a transporter protein comprising an amino acid sequence which has at least 95% identity with the amino acid sequence of SEQ ID NO:189 (T196_PtrPUP3_80).

11. The recombinant microbial host cell of any of the preceding claims, wherein the microbial cell is a fungus cell.

12. The recombinant host cell of any of the preceding claims, wherein the recombinant cell is a yeast cell.

13. The recombinant host cell of claim 12, wherein the yeast cell is a *Saccharomyces cerevisiae* cell.

14. A method of producing *in vivo* a thebaine derivative or an oripavine derivative in a cell culture, comprising culturing the host cell of any of the preceding claims in the cell culture, under conditions;
(A): in which at least one heterologously expressed enzyme of claim 1 capable of acting on reticuline or a derivative thereof to produce thebaine and/or oripavine is expressed and thereby present *in vivo* in the host cell; and
(B): in which thebaine and/or oripavine is present *in vivo* in the host cell; and
(C): wherein the thebaine or oripavine of item (B) *in vivo* is converted into a thebaine derivative and/or an oripavine derivative due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production a thebaine derivative or an oripavine derivative.

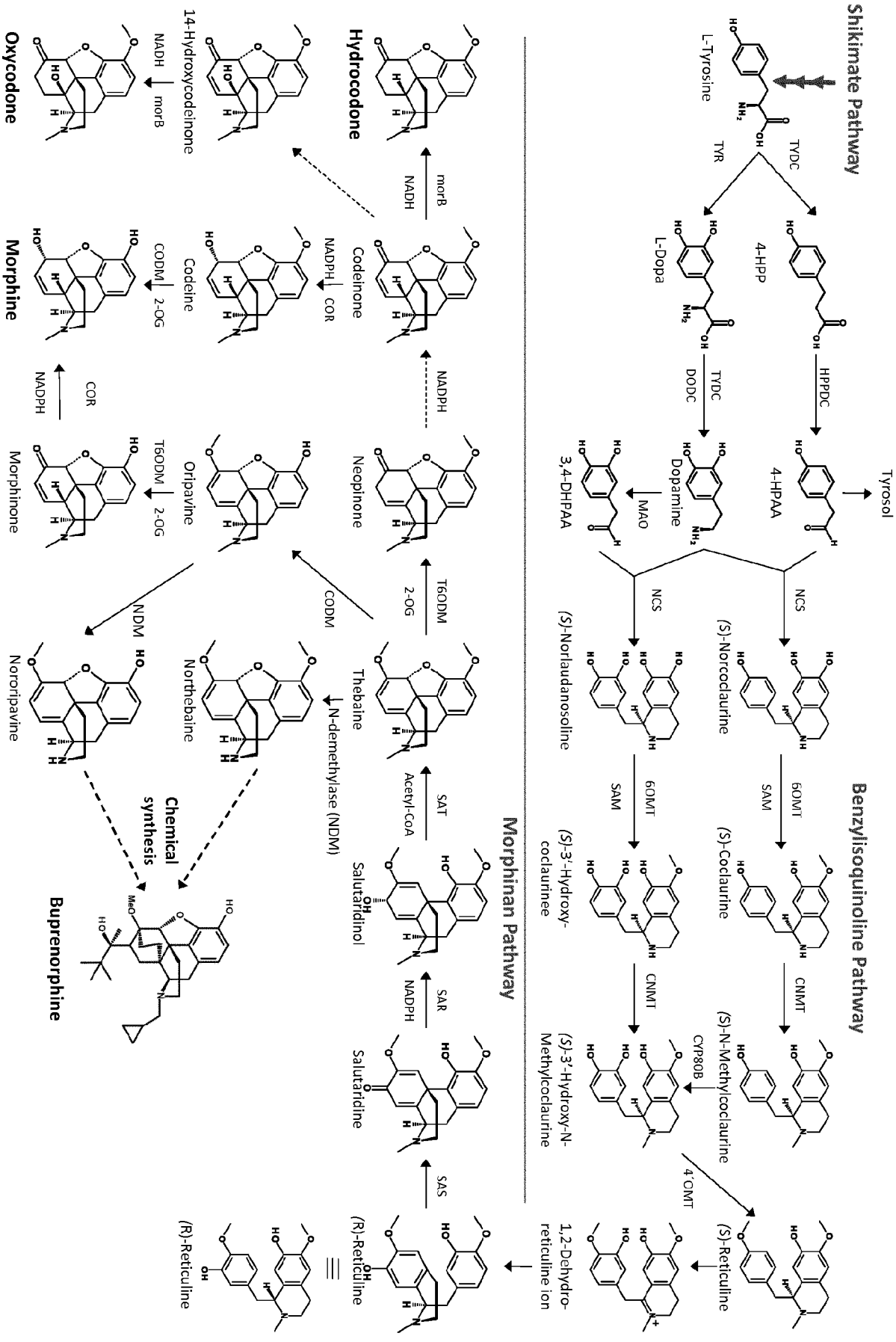
15. The method of claim 14, wherein the method is a method of *in vivo* producing neopinone, oripavine, northebaine, nororipavine or morphinone in a cell culture, comprising culturing the host cell of any of the preceding claims in the cell culture, under conditions;
(A): in which at least one heterologously expressed enzyme of claim 1 capable of acting on reticuline or a derivative thereof to produce thebaine and/or oripavine is expressed and thereby present *in vivo* in the host cell; and
(B): in which thebaine and/or oripavine is present *in vivo* in the host cell; and
(C): wherein the thebaine and/or oripavine of item (B) *in vivo* is converted into neopinone, oripavine, northebaine, nororipavine or morphinone due to the presence of the heterologously expressed enzyme of item (A) in order to thereby get *in vivo* production of neopinone, oripavine, northebaine, nororipavine or morphinone.

16. The method of *in vivo* producing a thebaine derivative or an oripavine derivative in a cell culture of claims 14 or 15, wherein the *in vivo* production a thebaine derivative or an oripavine derivative in

item (C) is an increased conversion of thebaine and/or oripavine relative to an otherwise identically performed method using an otherwise identical control host cell that is not heterologously expressing at least one functional transporter protein of any of the preceding claims.

17. A method of producing an opioid compound of interest, comprising first performing *in vivo* production of a thebaine derivative or an oripavine derivative (such as e.g. neopinone, oripavine, northebaine, nororipavine or morphinone) according to any of claims 14 to 16 followed by suitable *in vivo* and/or *in vitro* synthesis steps in order to obtain one or more opioid compounds of interest selected from heroin, morphine, codeine, oripavine, oxycodone, hydrocodone, hydromorphone, oxymorphone, buprenorphine, naltrexone, naloxone, nalmefene, noroxymorphone and nalbuphine.

Figure 1


Shikimate Pathway
L-Tyrosine
TYR
TYDC
4-HPP
L-Dopa
HPPDC
TYDC
DODC
Tyrosol
4-HPAA
Dopamine
MAO
3,4-DHPAA
Benzylisoquinoline Pathway
NCS
(S)-Norcoclaurine
6OMT
SAM
(S)-Coclaurine
CNMT
(S)-N-Methylcoclaurine
CYP80B
4'OMT
(S)-Reticuline
NCS
(S)-Norlaudanosoline
6OMT
SAM
(S)-3'-Hydroxy-coclaurinee
CNMT
(S)-3'-Hydroxy-N-Methylcoclaurine
1,2-Dehydro-reticuline ion
Morphinan Pathway
SAS
(R)-Reticuline
(R)-Reticuline
SAR
NADPH
Salutaridine
SAT
Acetyl-CoA
Salutaridinol
N-demethylase (NDM)
Thebaine
T6ODM
2-OG
Neopinone
NADPH
Hydrocodone
morB
NADH
14-Hydroxycodeinone
NADH
morB
Oxycodone
Northebaine
NDM
Norcodeine
CODM
Codeine
NADPH
COR
Codeinone
CODM
2-OG
Morphine
Oripavine
T6ODM
2-OG
Morphinone
NADPH
COR
Chemical synthesis
Buprenorphine

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/077548

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/80 C12P17/18
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/075670 A1 (ANTHEIA INC [US]) 26 April 2018 (2018-04-26) cited in the application Abstract; paragraphs 284, 286, 303, 340, 352; examples; claims -----	1-17
Y	S. GALANIE ET AL: "Complete biosynthesis of opioids in yeast", SCIENCE, vol. 349, no. 6252, 4 September 2015 (2015-09-04), pages 1095-1100, XP055317485, ISSN: 0036-8075, DOI: 10.1126/science.aac9373 cited in the application the whole document -----	1-17
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 November 2019	22/11/2019

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	NOBUKAZU SHITAN ET AL: "Alkaloid transporters in plants", PLANT BIOTECHNOLOGY, vol. 31, no. 5, 25 December 2014 (2014-12-25), pages 453-463, XP55639000, JP ISSN: 1342-4580, DOI: 10.5511/plantbiotechnology.14.1002a the whole document	1-17
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/077548

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