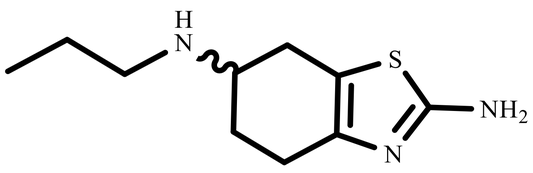
The biocatalytic approach to the preparation of Pramipexole

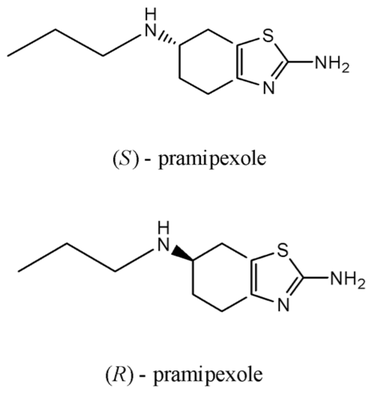
Benedetta Guidi[a*], Patrizia Ferraboschi[a], Samuele Ciceri[a], Martina Letizia Contente[b], Valerio De Vitis[b] and Francesco Molinari[b].

[a] Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy.
[b] Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Milan, Italy.

INTRODUCTION

PRAMIPEXOLE



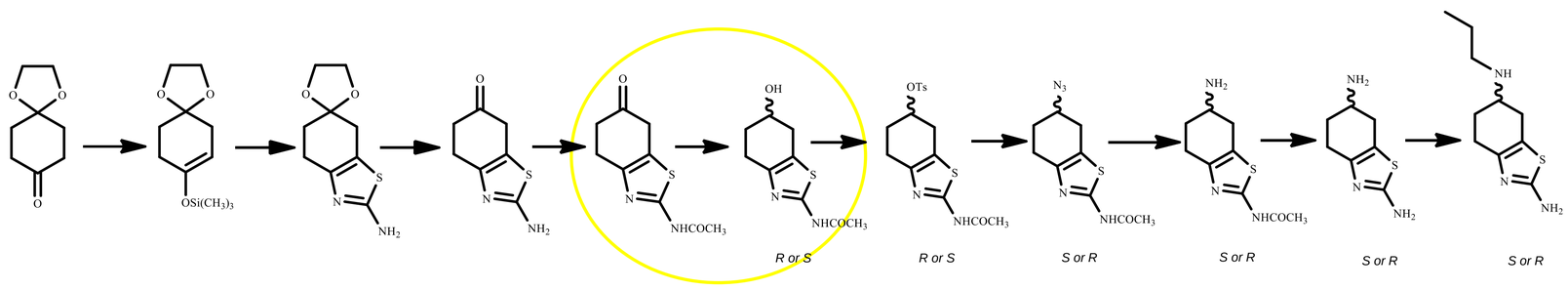


Two enantiomers of the same molecule show a different biological activity.

(*S*)-Pramipexole is the most prescribed dopamine agonist in the ANTI-PARKINSON THERAPY.

(*R*)-Pramipexole is currently in clinical development for the treatment of AMYOTROPHIC LATERAL SCLEROSIS (ALS).

CHEMICAL SYNTHESIS DEVELOPED

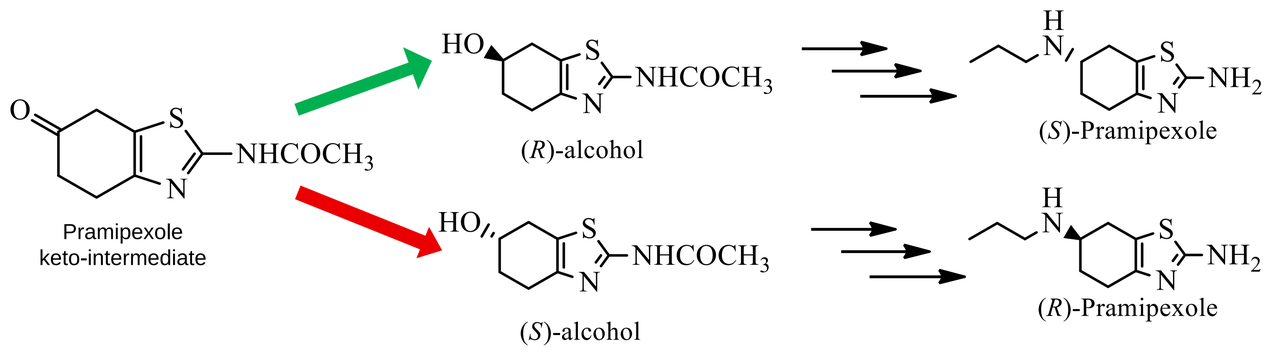


In this new synthetic way reduction of the prochiral ketone represents the key point to achieve an enantomerically pure pramipexole.

The biocatalytic preparation of optically pure chiral building blocks is a valid alternative to conventional chemical methods.

AIM OF WORK

Through screening for ketoreductase activity we are going to identify biocatalysts with marked enantioselectivity, in order to obtain both optically pure alcohols.



RESULTS

Saccharomyces cerevisiae

BEST BIOTRANSFORMATION CONDITIONS

- Phosphate buffer 100 mM, pH 7
- *S. cerevisiae* liophilized whole cells 14 g/L
- Saccarose 50 g/L
- Substrate: keto-intermediate 2,5 g/L
- eptane/water phase 1:1

Under stirring at 30°C, 180 rpm.

Experimental conditions	Time (h)	Conversion (%)	(*R*) e.e. (%)
buffer pH 7	18	100	92
buffer pH 7/*n*-heptane 1:1	18	100	94
buffer pH 7/*n*-heptane 1:1	16	87	>98

* Determinated by HPLC on chiral stationary phase.

RESULTS

MARINE YEASTS

Screening on twenty marine yeast strains, from MaCuMBA[1] European project collection[2].

PRELIMINARY BIOTRANSFORMATION CONDITIONS

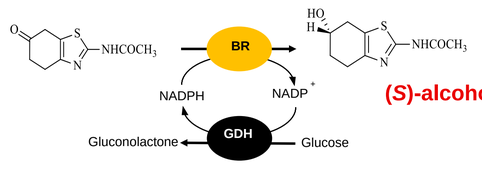
- Growing cells after 48h in YPD+3% NaCl
- Glucose 50 g/L
- Substrate: keto-intermediate 1 g/L (isopropanol)

Under stirring at 30°C,180 rpm.

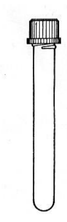
Microorganisms	Identification number	T (h)	Conversion *	e.e.*	Configuration
Meyerozyma guilliermondii strain	1	24	100%	7,5%	*R*
Meyerozyma guilliermondii strain	2	24	100%	5%	*R*
Meyerozyma guilliermondii strain	3	24	100%	1,3%	*R*
Rhodotorula mucilaginosa strain	4	24	100%	40%	*S*
Meyerozyma guilliermondii strain	5	24	100%	2%	*R*
Meyerozyma guilliermondii strain	6	24	100%	1%	*R*
Not sequenced	7	24	84%	26%	*R*
Rhodotorula mucilaginosa strain	8	24	100%	45%	*S*
Rhodotorula mucilaginosa strain	9	24	100%	0,15%	*S*
Rhodotorula mucilaginosa strain	10	24	100%	42%	*S*
Rhodotorula mucilaginosa strain	13	24	100%	62%	*S*
Rhodotorula mucilaginosa strain	14	24	98%	50%	*S*
Rhodotorula mucilaginosa strain	15	24	100%	56%	*S*
Rhodotorula mucilaginosa strain	16	24	100%	64%	*S*
Rhodotorula mucilaginosa strain	17	24	100%	48%	*S*
Rhodotorula mucilaginosa strain	18	24	100%	38%	*S*
Rhodotorula mucilaginosa strain	19	24	100%	48%	*S*
Rhodotorula mucilaginosa strain	20	24	100%	50%	*S*
Rhodotorula mucilaginosa strain	21	24	100%	50%	*S*
Rhodotorula mucilaginosa strain	22	24	100%	49%	*S*

* Determinated by HPLC on chiral stationary phase.

BENZIL REDUCTASE[3] from *Pichia glucozyma* [4]



BIOTRANSFORMATION CONDITION



- Benzil reductase
- Glucose dehydrogenase
- Substrate: keto-intermediate 1 g/L
- Glucose
- Tris HCl buffer 50 mM, pH8
- $NADP^+$

Under stirring at 30°C for 24h, 150 rpm.

	0 h	2,5 h	4 h
Conversion*	15%	67%	75%
(*S*) e.e.*	43%	53%	86%

* Determinated by HPLC on chiral stationary phase.

CONCLUSIONS

- The common baker yeast *Saccharomyces cerevisiae* afforded with yields of 80% optically pure (*R*)-alcohol precursor of the anti-Parkinson (*S*)-pramipexole. The optically pure (*S*)-alcohol, required for the synthesis of (*R*)-pramipexole, under investigation for the treatment of ALS, has been isolated with low yields by inversion of configuration from (*R*)-alcohol.
- Twenty marine yeast strains were screened for ketoreductase activity with the aim of reducing the ketone with a stereochemical outcome opposite than *S.cerevisiae,* affording (*S*)-alcohol. In particular, *Rhodotorula mucillaginosa* strains gave (*S*)-alcohol with an e.e. ranging from 38% to 64%.
- Application of a novel benzil reductase from *Pichia glucozyma,* thanks to co-factor recycling system, showed that isolated enzyme is able to produce (*S*)-alcohol with e.e. 86% .

FUTURE DEVELOPMENTS

- Optimisation of marine yeasts biotransformation conditions .
- Screening of new microorganisms.
- Isolation, cloning and expression of the best performing proteins from microorganism screened active on the suitable ketone.
- Setting up a continuous baker yeast catalysed biotransformation based on Flow Chemistry technique.

REFERENCES

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* benedetta.guidi@unimi.it