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Triazole-indole hybrid molecules as antifungal agents: Design, synthesis and biological activity, and beyond

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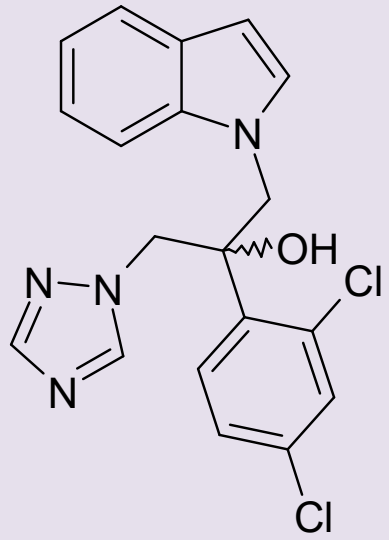
Triazole-indole hybrid molecules as antifungal agents: Design, synthesis and biological activity, and beyond

$C_{19}H_{16}Cl_2N_4O$

MW = 387.26

rac **8g***



Structural diversity explored
Synthetic process optimized (MW)
SAR analysis

***C. albicans* CA98001**
(-)-**8g** (MIC* = **0.000256 mg/mL**)
(+)-**8g** (MIC = 0.023 mg/mL)
fluconazole (MIC = 0.020 mg/mL)

8g active in vivo three times a day

*(-/+)-2-(2,4-dichlorophenyl)-3-(*1H*-indol-1-yl)-1-(*1H*-1,2,4-triazol-1-yl)-propan-2-ol

*MIC = IC_{80}

Abstract: Invasive fungal infections have increased in frequency and severity over the last twenty years as a result of an increasing number of immunocompromised hosts due to cancer chemotherapy, organ and bone marrow transplantation, or therapy against autoimmune and inflammatory disorders. Candida species (spp.) are among the most common pathogens. *Candida albicans* is the main cause of candidiasis. In addition non-*albicans Candida* spp. are becoming more and more involved in nosocomial infections. The emergence of resistance to conventional treatments (e.g. fluconazole) make healing successes weaker. It is therefore urgent to continue efforts to develop new antifungal agents. A series of 2-aryl-3-azolyl-1-indolyl-propan-2-ols was designed as new analogs of fluconazole by replacing one of its two triazole moieties by an indole scaffold. Two different chemical pathways were developed; the first one included seven steps and the second one only three. The pharmacomodulation works have enabled us to identify a molecule with a strong biological impact on fungi. Numerous experiments progressively confirmed the high potential of this hybrid molecule as antifungal agent. In this presentation, all aspects of medicinal chemistry will be addressed.

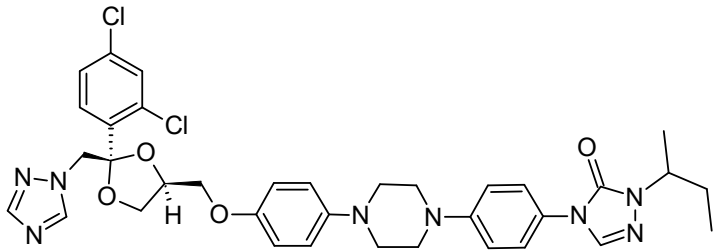
Keywords: triazole; indole; antifungal agents; molecular modeling; microwave irradiation; X-ray crystallography; Candida species; cytochromes P450

Introduction – Fungi and molecular target

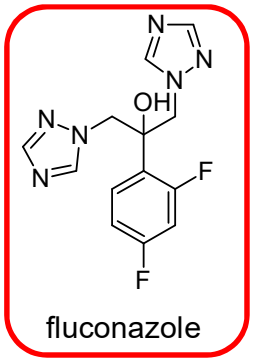
- Opportunistic fungal infections are **dramatically increased**…
- Emergence of ***C. krusei*** and ***C. glabrata*** infections
- Mortality of established fungal diseases remains **high**
- Key compound for fungi: **Ergosterol** (fungal cell membrane)
- Key enzyme in ergosterol biosynthesis: **14α-lanosterol demethylase** (**CYP51**)

Introduction – Conazoles

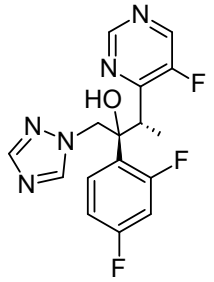
- **Structures of the main triazole antifungal agents targeting CYP51**



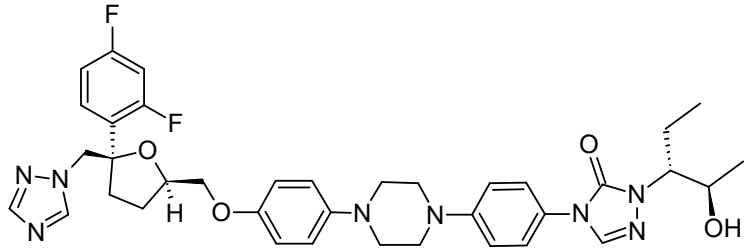
itraconazole



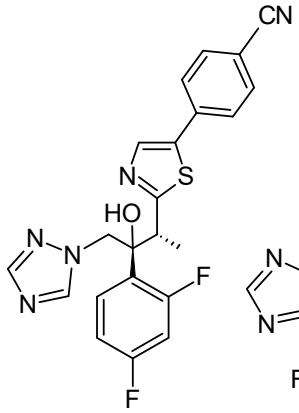
fluconazole



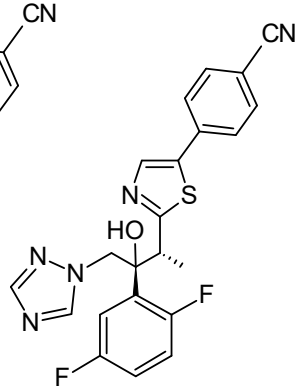
voriconazole



posaconazole



ravuconazole



isavuconazole

Introduction – Development of new therapeutic entities (NTE)

- **Why a need for alternative drugs is important…**

Toxicity of actual antifungal drugs (nephrotoxicity…)

Emerging resistance to azoles (*ERG11* gene)
Emerging resistance implicating CaMdr1p and CaCdr1p

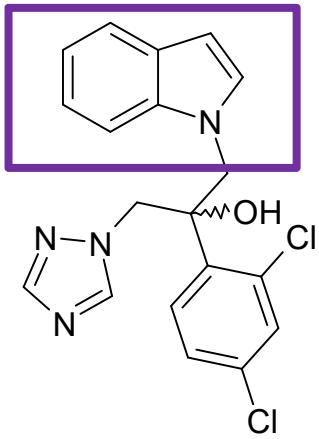
Increasing number of immunocompromised hosts
Increasing number of invasive fungal nosocomial infections

Novel, safe and effective antifungal agents are clearly needed…

Introduction – Development of new fluconazole analogues

- ❑ Access to 3-(indol-1-yl)-2-phenyl-1-(triazol-1-yl)propan-2-ols
- ❑ Synthetic process optimized by microwave (MW) irradiations
- ❑ Chiral separation and X-ray studies
- ❑ *In vitro* and *in vivo* evaluations
- ❑ Selectivity on cytochrome P450 enzymes
- ❑ Mechanism of action and Molecular studies

Modulation of fluconazole: Replacement of one triazole by indole



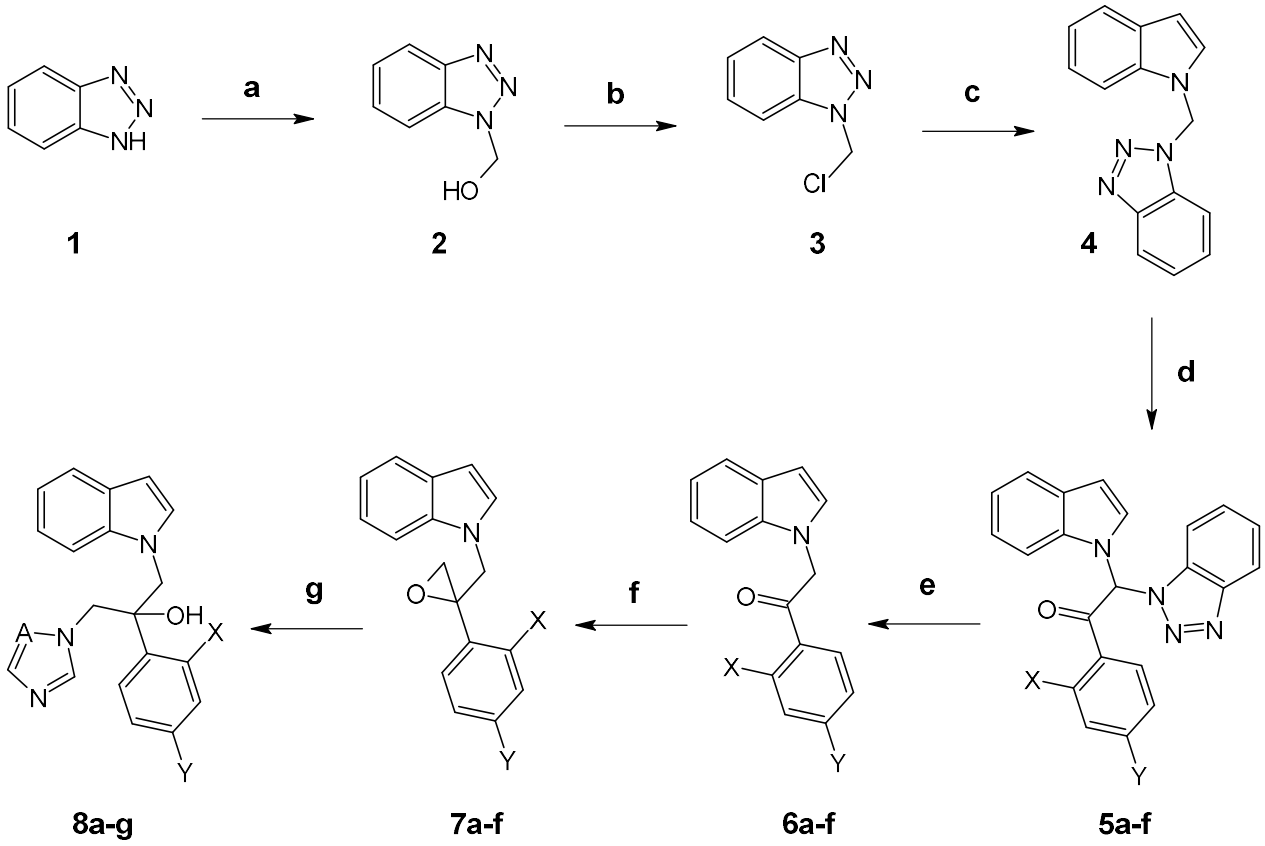
Results and discussion – Synthesis of fluconazole analogues

❑ Linear synthesis

Seven steps

X = H, Cl, F
Y = Br, Cl, F, CF_3
A = CH, A

8g: X,Y = Cl
A = N

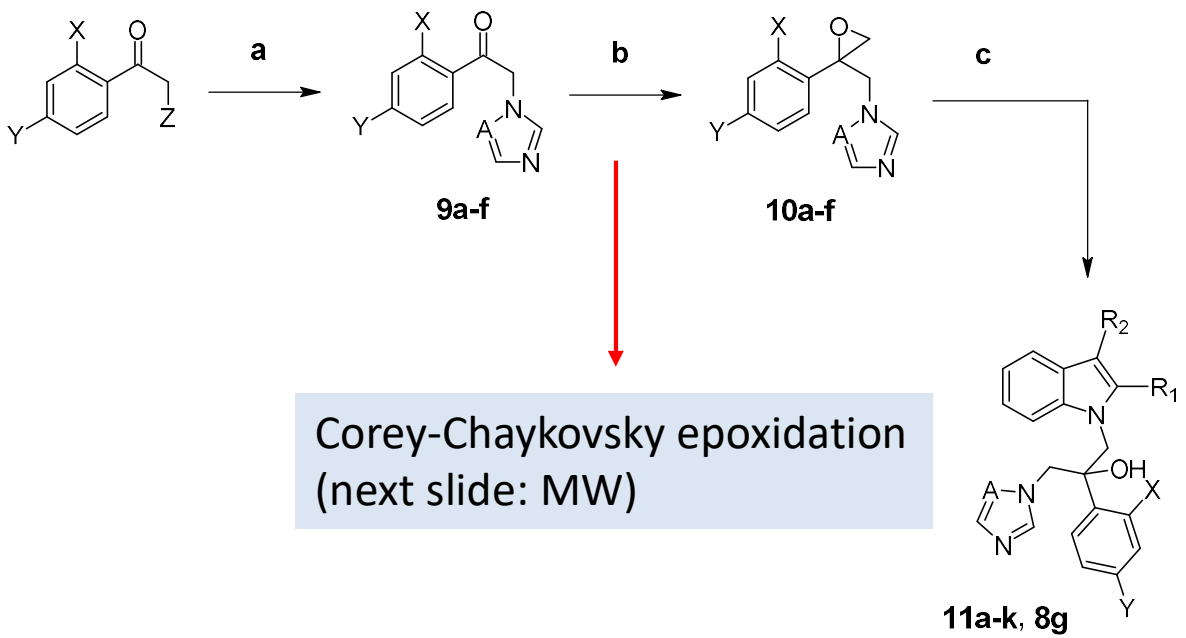


Results and discussion – Synthesis of fluconazole analogues

❑ **Shorter process**

Only three steps

X = H, Cl, F
Y = Br, Cl, F, CF_3
A = CH, A
R_1, R_2 = H, CH_3

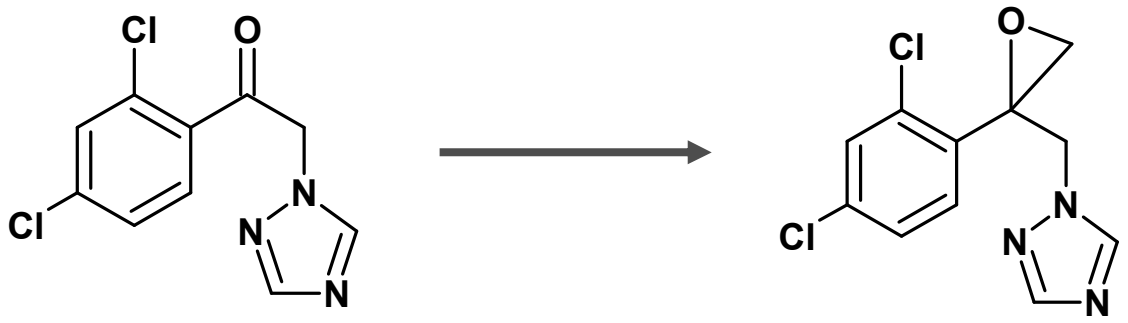


Corey-Chaykovsky epoxidation
(next slide: MW)

Reagents and conditions: **a.** K_2CO_3, imidazole or *1H*-1,2,4-triazole, CH_3CN, **MW** 85 °C, 50 W, 50 min; **b.** $NaOH_{aq}$ 20%, TMSOI, CH_2Cl_2, reflux, 72 h; **c.** NaH, indole derivative, DMSO, rt, 12 h.

Results and discussion – Synthesis of fluconazole analogues

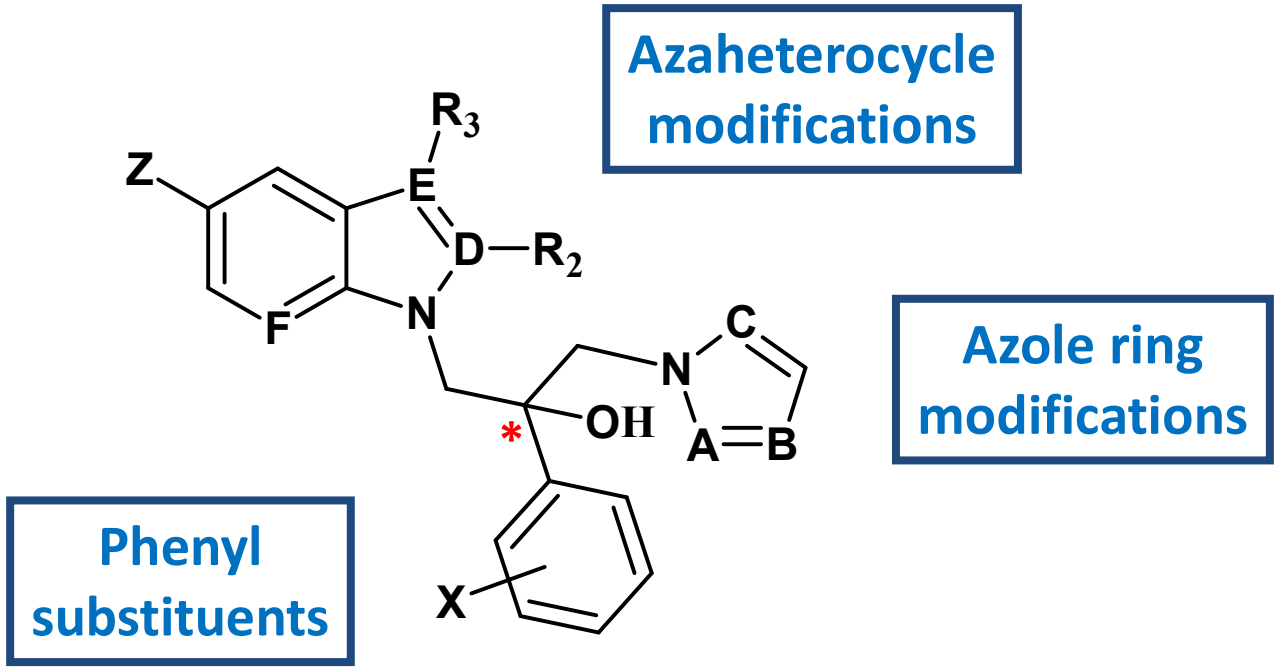
❑ **Corey epoxidation**



solvent	base	Δ (°C)	time	yield (%)
DMSO	NaH	r.t.	4 days	74
CH_2Cl_2	NaOH	50	2 days	78
CH_2Cl_2	NaOH	MW, 50	150 min	75

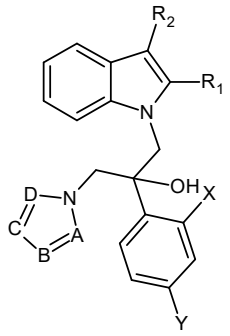
Results and discussion – Structural diversity

- Starting scaffold: Indole

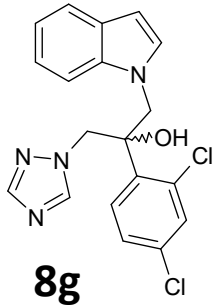


Results and discussion – Pharmacomodulation

❑ SAR study



Best combination



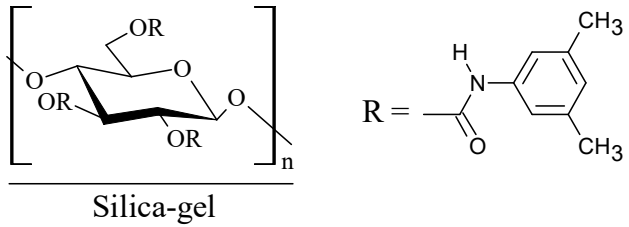
8g

Compd	A	B	C	D	R_1	R_2	X	Y	MIC [1] (μg/mL) *C. albicans* CA98001
8a	CH	CH	N	CH	H	H	H	F	0.03000
8b	CH	CH	N	CH	H	H	H	Cl	0.02000
8c	CH	CH	N	CH	H	H	H	Br	0.02000
8d	CH	CH	N	CH	H	H	F	F	0.00035
8e	CH	CH	N	CH	H	H	Cl	Cl	0.06200
11e	CH	CH	N	CH	H	H	H	CF_3	0.23000
11a	N	CH	N	CH	H	H	H	F	0.21000
11b	N	CH	N	CH	H	H	H	Cl	0.02400
11c	N	CH	N	CH	H	H	H	Br	0.02700
11d	N	CH	N	CH	H	H	F	F	0.01980
8g	N	CH	N	CH	H	H	Cl	Cl	0.000259
(+)-(*R*)-8g	N	CH	N	CH	H	H	Cl	Cl	0.02300
(-)-(*S*)-8g	N	CH	N	CH	H	H	Cl	Cl	0.000256
8f	N	CH	N	CH	H	H	H	CF_3	0.00900
11f	N	CH	N	CH	CH_3	H	F	F	0.02200
11g	N	CH	N	CH	CH_3	H	Cl	Cl	0.00580
11h	N	CH	N	CH	H	CH_3	F	Cl	0.00110
11i	N	CH	N	CH	H	CH_3	Cl	Cl	0.00700
11j	N	CH	N	CH	CH_3	CH_3	F	F	0.15700
11k	N	CH	N	CH	CH_3	CH_3	Cl	Cl	1.24600
11l	N	N	CH	CH	H	H	Cl	Cl	> 100
11m	N	CH	CH	N	H	H	Cl	Cl	> 100
KTC									0.00500
FLC									0.02000

[1] Minimum inhibitory concentration (MIC = IC_{80}, μg/mL)

Results and discussion – Chiral HPLC of racemic 8g

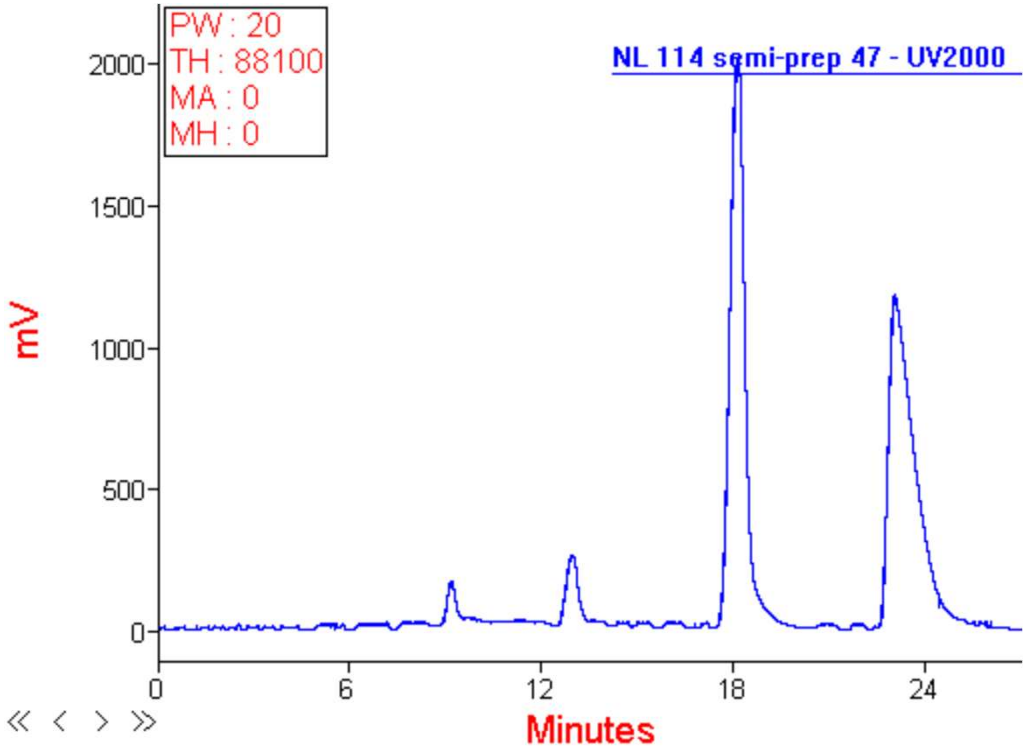
❑ Chiral Stationary Phase



Chiralcel OD-H column

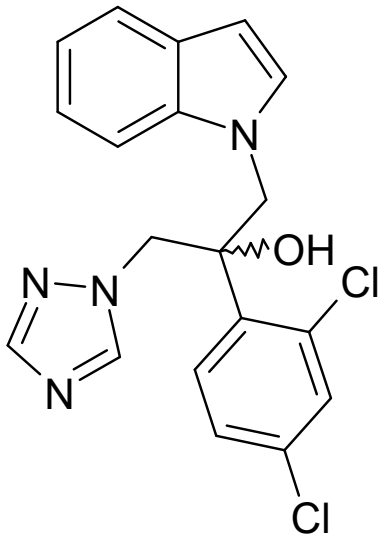
Mobile Phase:

CH_3CN/MeOH (85/15) +
0.1% diethylamine



Results and discussion – Chiral separation of racemic 8g

❑ **Optical rotation values**



rac **8g**

(+)-8g

$[\alpha]_D^{24}$ = 46.2 (c=2.6, $CHCl_3$)

(-)-8g

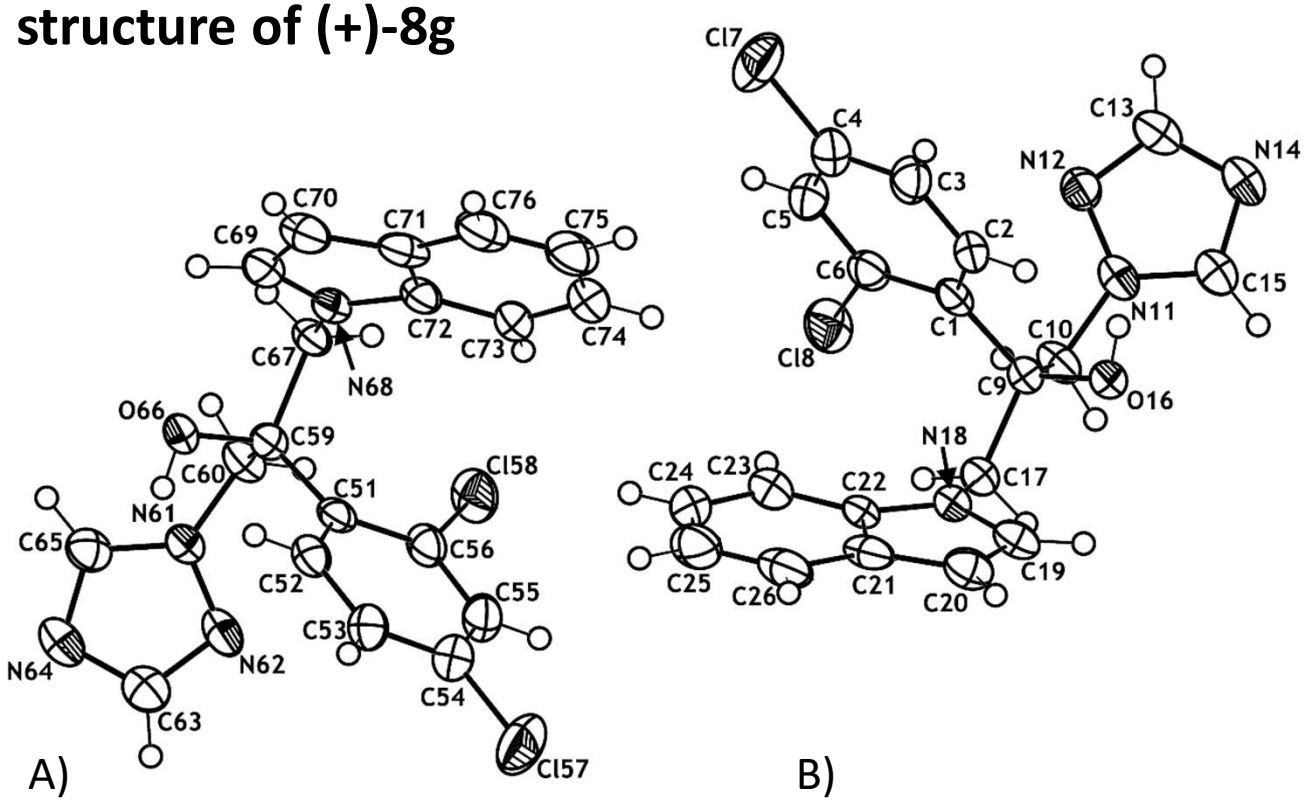
$[\alpha]_D^{24}$ = - 42.4 (c=2.1, $CHCl_3$)

Results and discussion – X-ray studies of rac 8g

❑ **View of the crystal structure of (+)-8g**

Two independent molecules, designated as A and B, were found in the asymmetric crystallographic unit

enantiomer (+)-(*R*)-**8g**

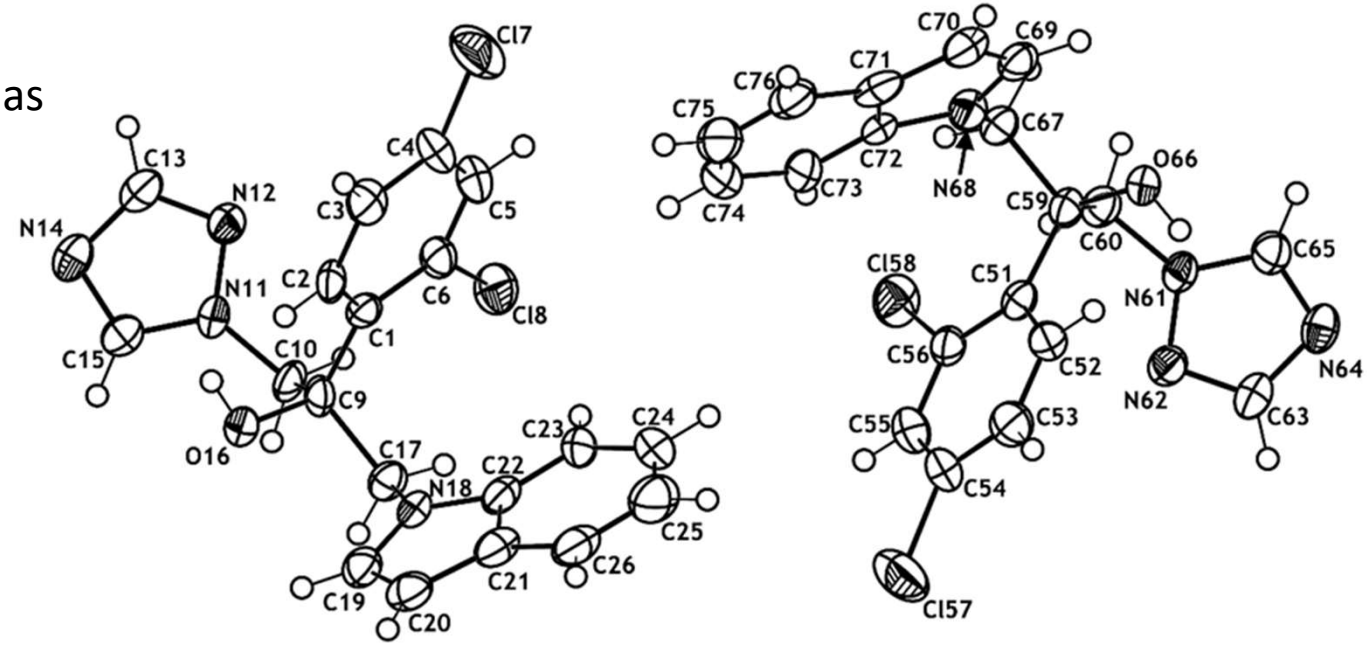


Results and discussion – X-ray studies of rac 8g

❑ View of the crystal structure of (-)-8g

Two independent molecules, designated as A and B, were found in the asymmetric crystallographic unit

enantiomer (-)-(*S*)-**8g**



A) B)

Results and discussion – Biological evaluation (*in vitro*)

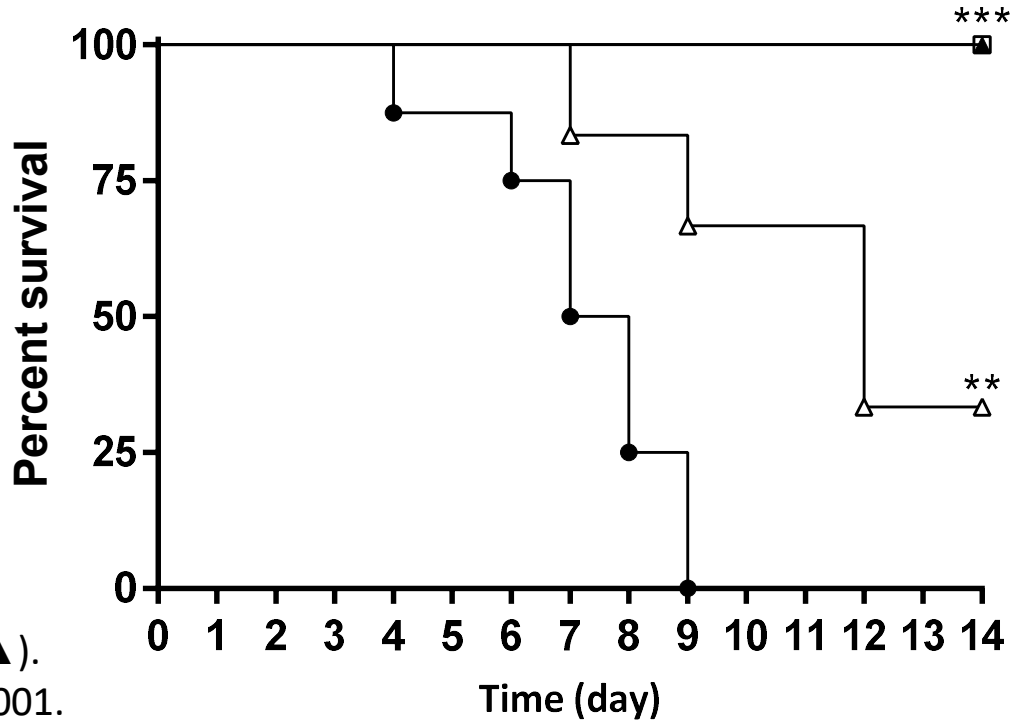
❑ **Antifungal susceptibilities of *Candida* spp. to rac 8g**

Species (no. of isolates)	Antifungal agent	MIC (µg/mL)		
		Range	Geometric mean	MIC_{90}
C. albicans (27)	rac **8g** fluconazole	< 0.016 - 4 < 0.125 - > 64	0.038 0.696	**0.5** 8
C. glabrata (13)	rac **8g** fluconazole	< 0.06 - 0.5 0.016 - > 64	0.06 4	**0.25** 64
C. krusei (15)	rac **8g** fluconazole	0.016 - 1 8 - > 64	0.109 23	**0.125** 64
C. parapsilosis (20)	rac **8g** fluconazole	< 0.016 - 0.125 < 0.125 - > 64	0.029 0.661	**0.0625** 8
Candida spp. (81)	rac **8g** fluconazole	< 0.016 - 4 < 0.125 - > 64	0.048 2	**0.5** 64

Results and discussion – Biological evaluation (*in vivo*)

- **Systemic candidiasis model**

Importance to spread out the daily dose of **8g**



Fluconazole (3 x 5 mg/kg *per os*, □) or **8g** (2 x 30 mg/kg ip, Δ; **3 x 20 mg/kg ip**, ▲). Control group (●). ** $p < 0.01$; *** $p < 0.001$.

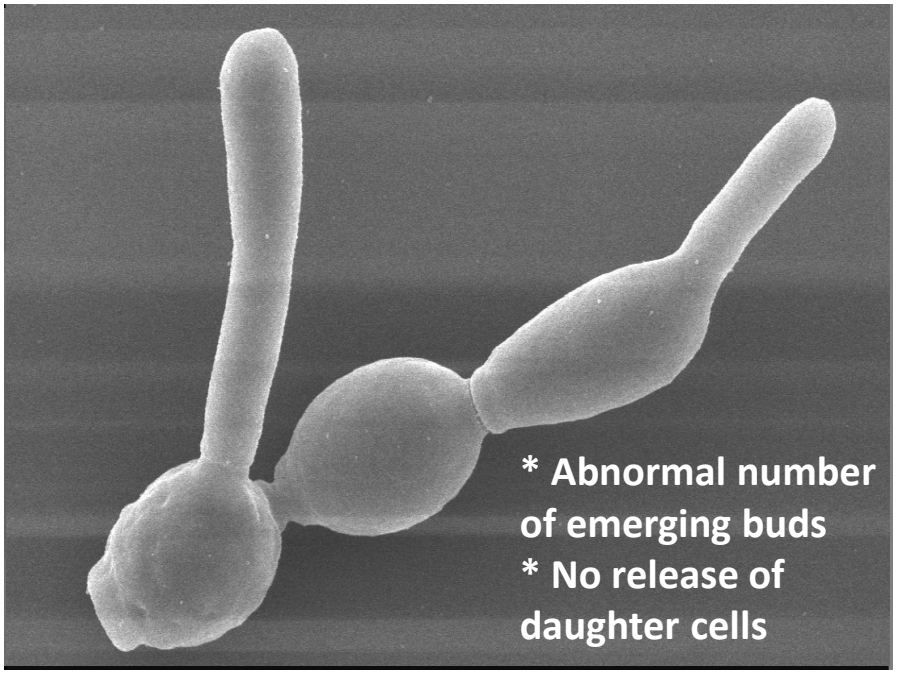
Results and discussion – Mechanism of action

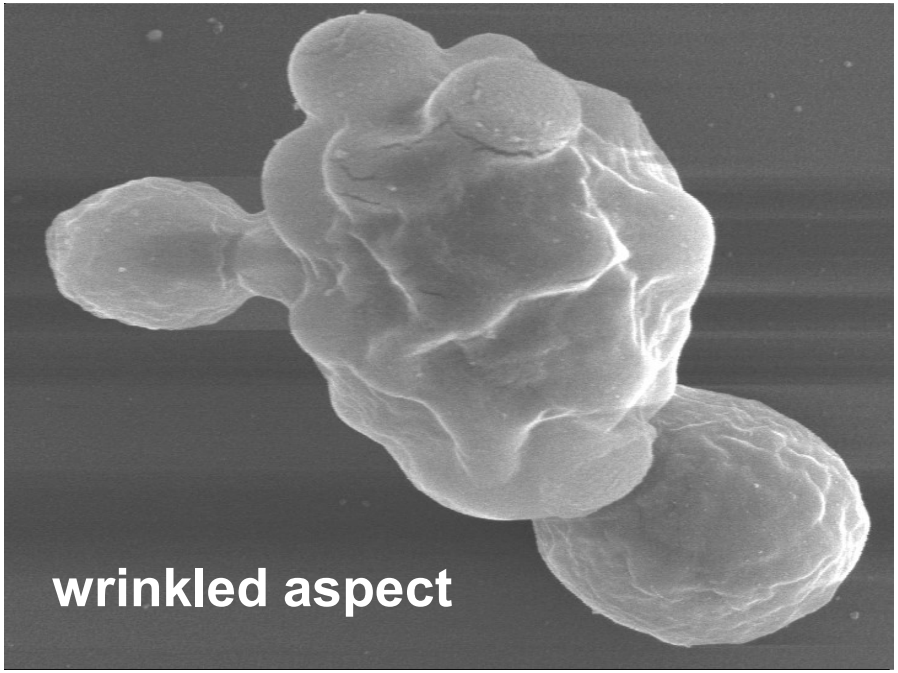
❑ Study on sterol profile

		CAAL93			*CAKR7*			*CAGL2*	
		control	**8g**	FLU	control	**8g**	FLU	control	**8g**
	R.T.		4 ng/mL	4 µg/mL		4 µg/mL	4 µg/mL		4 µg/mL
zymosterol	9.34	4.8	-	-	2.4	-		-	-
24-ethyl-cholesta-5,7,22-trienol	9.49	-	-	8.5	-	-	-	-	-
ergosterol	**9.55**	**69.4**	**21.6**	**-**	**83.1**	**60.1**	**84.3**	**78.4**	**41.6**
14α-methylepisterol	9.71	-	13.5	30.7	-	3.5		-	2.6
ergosta-7,22-dienol	9.73	5.5	-	-	-	-	-	-	-
fecosterol	9.80	-	-	-	1.9	-	1.8	-	-
14α-methylfecosterol	9.973	-	10.0	10.8	-	4.9		-	3.5
ergostadien-3β-ol (5,7 ou5,8)	10.05	-	-	-	1.1	-	1.1	3.7	2.2
episterol	10.26	3.4	-	-	-	-	-	1.9	-
14α-3,6 diol	**10.41**	**-**	**-**	**-**	**-**			**-**	**23.4**
lanosterol	**10.55**	**5.2**	**35.4**	**34.2**	**0.7**	**24.2**	**1.8**	**3.6**	**-**
obtusifoliol	10.60	-	-	-	-	-	-	-	23.8
4,4-dimethylcholesta-8,24-dienol	10.80	1.4			1.2		1.1	2.7	
eburicol	11.20	-	19.0	15.7	-	3.9	-	-	0.7

Results and discussion – Scanning electronic microscopy

❑ Fongistatic effect

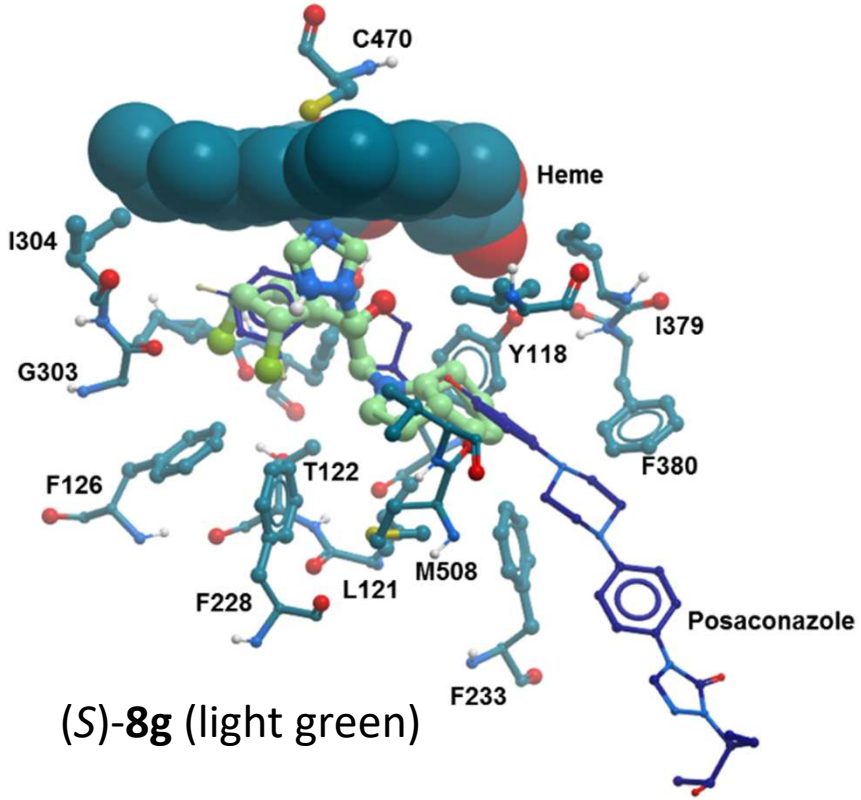




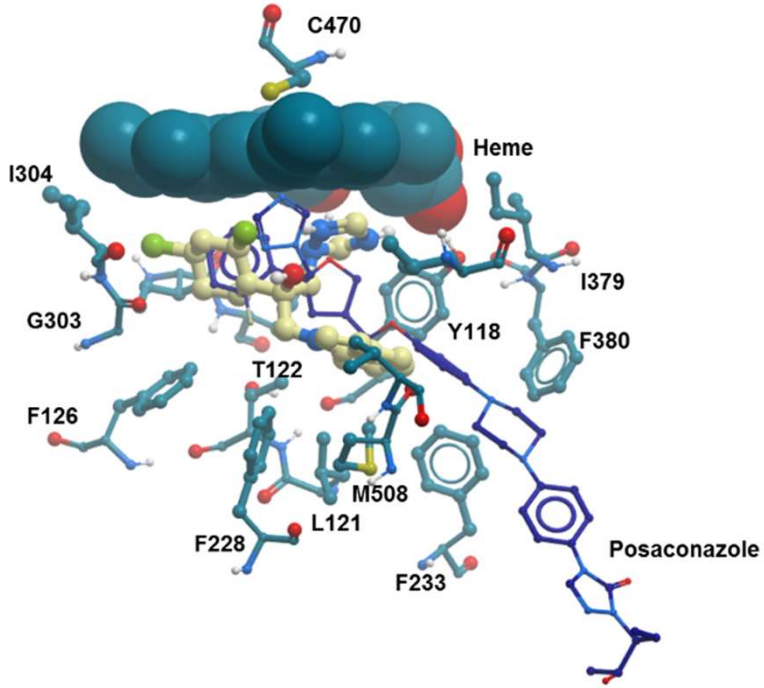
Candida albicans was treated during 10 h with **racemic 8g** (c = 25 nM)

Results and discussion – Molecular docking

- **Binding pose of each enantiomer of 8g** (PDB ID: 5FSA, CaCYP51, co-crystallized with posaconazole)



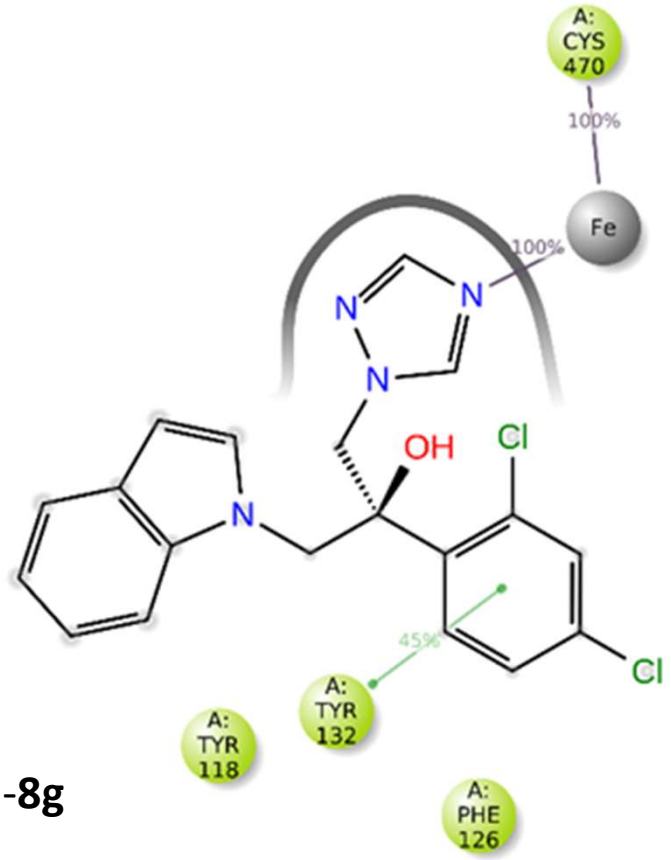
(*S*)-**8g** (light green)



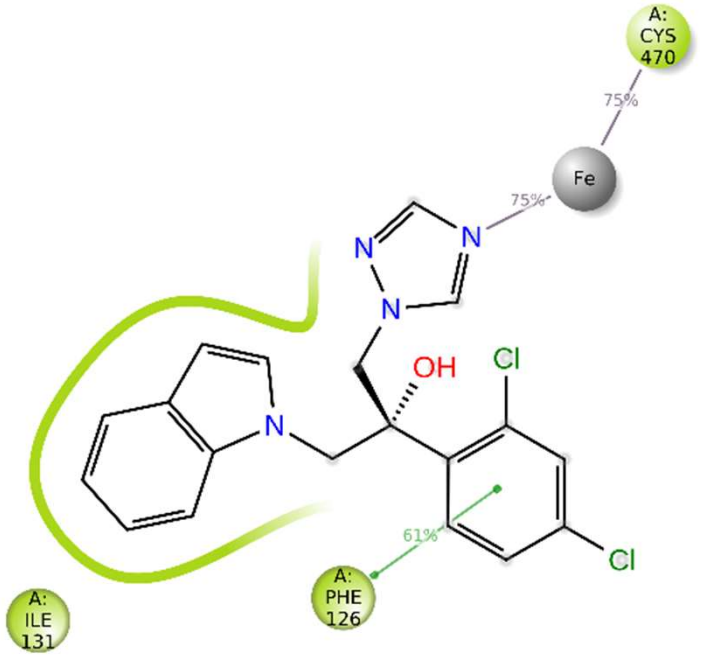
(*R*)-**8g** (cream)

Results and discussion – Molecular docking

- **Molecular dynamics simulation** (ligand atom interactions with CaCYP51 - 5FSA)



(*S*)-**8g**



(*R*)-**8g**

Results and discussion – Selectivity profile

❑ **Selectivity of compound 8g and its enantiomers on a panel of CYP450s**

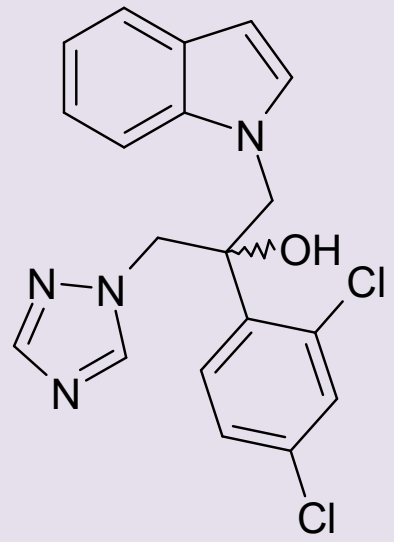
Compd	CYP19 IC_{50} (μM) (% inhib.)	CYP17 IC_{50} (μM) (% inhib.)	CYP26A1 IC_{50} (μM)	CYP11B1 IC_{50} (μM) (inhib. effect)	CYP11B2 IC_{50} (μM) (inhib. effect)
8g	- (27)	- (< 10%)	-	- no inhib.	- slight inhib.
(+)-(*R*)-8g	- (51)	- (< 5% inhib.)	34	- no inhib.	- slight inhib.
(-)-(*S*)-8g	- (72)	- (< 5% inhib.)	18	- no inhib.	- no inhib.
aminoglutethimide	29.75	-	-	-	-
fadrozole	0.030	not active	-	-	-
anastrozole	0.163	-	-	-	-
letrozole	0.025	-	-	-	-
liarozole	-	-	7	-	-
BW19	-	0.15	-	-	-
ketoconazole	-	4.5	10	-	-

Conclusions – Perspectives

$C_{19}H_{16}Cl_2N_4O$

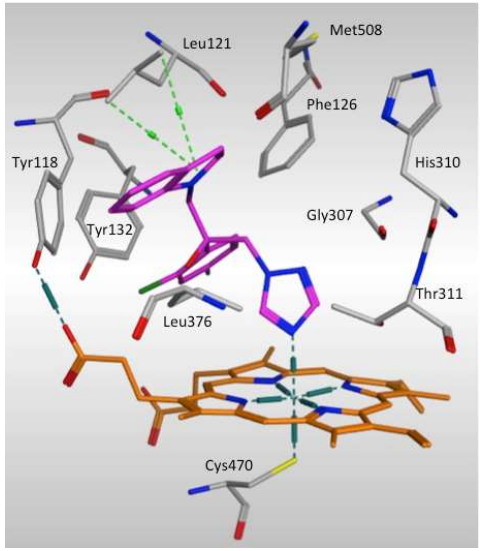
MW = 387.26

rac **8g**
(+)-(*R*)-**8g**
(-)-(*S*)-**8g**



- Toxicity on **MRC5 cells**
  8g: IC_{50} = 35 µM
  (+)-(*R*)-**8g**: IC_{50} = 32 µM
  (-)-(*S*)-**8g**: IC_{50} = 30 µM

- 3D Image of (*S*)-**8g** (magenta) ligand-CaCYP51 complex after **MD simulation**



- **Active** on Candida spp. +++
- **Not active** on *Aspergillus fumigatus*
- **New** pharmacomodulation studies series propanol, butanol…
- New biological investigations on **neglected diseases**

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