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REVIEW ARTICLE

Targeting *Malassezia* species for Novel Synthetic and Natural Antidandruff Agents

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Abstract: *Malassezia* spp. are lipophilic yeasts not only present in the normal skin microflora, but also responsible of skin-related diseases (pityriasis versicolor, seborrheic/atopic dermatitis and dandruff) as well as systemic fungal infections in humans and animals. Their treatment and eradication are mainly based on old azole drugs, which are characterized by poor compliance, unpredictable clinical efficacy, emerging resistance and several side effects. These drawbacks have prompted the research toward novel synthetic and natural derivatives/nanomaterials targeting other pivotal enzymes/pathways such as carbonic anhydrase (MgCA) and lipases, alone or in combination, in order to improve the eradication rate of this fungus. This review accomplished an update on this important topic dealing with the latest discoveries of synthetic scaffolds and natural products for the treatment of *Malassezia* spp.-related diseases, thus suggesting new opportunities to design innovative and alternative anti-dandruff drugs.

Keywords: Azoles, carbonic anhydrase inhibitors, dandruff, lipase inhibitors, *Malassezia* spp., natural inhibitors.

1. INTRODUCTION

Malassezia species are ubiquitous single-celled basidiomycetous yeasts, which are common elements of fungal microbiota of animal and human skin. Some species could produce hyphae when they become pathogenic. They are divided into 15 species of which 14 are lipid dependent. This genus includes *M. cuniculi*, *M. nana*, *M. slooffiae*, *M. caprae*, *M. pachydermatis*, *M. globosa*, *M. sympodialis*, *M. equina*, *M. dermatis*, *M. furfur*, *M. japonica*, *M. obtusa*, *M. restricta*, *M. yamatoensis* [1] and the recently discovered *M. arunalukei* [2]. *M. sympodialis*, *M. furfur*, *M. slooffiae*, *M. restricta* and *M. globosa* are the most commonly detected species in human skin [3]. Conversely, *M. pachydermatis*, a zoophilic species, is known to cause the majority of external otitis and seborrheic dermatitis cases in dogs [4]. Interestingly, there is evidence indicating its role also in systemic infections in hospitalized and parenterally fed human infants [5,6].

Studied by electron microscopy, *Malassezia* cells structurally show a thick and multilaminar cell wall, composed of chitin and an extremely high level of lipids (15–20%, *w*/*w*) greater than *Candida albicans* and *Saccharomyces cerevisiae*, with a characteristic invagination, a cell membrane, and vital organelles [7,8]. Its cell surface hydrophobicity (CSH) has been recognized as the main pathogenic factor contributing to drug resistance, reduction of the immune susceptibility, and development of inflammation [9].

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Recently, some authors elucidated the polysaccharide composition in the cell wall of *M. restricta*, determining its unusual and unique structure and alkali-resistance due to a specific content of 5% chitin, 20% chitosan, 5% β-(1–3)-glucan, and 70% β-(1–6)-glucan [10]. The hydrophobic organization makes *Malassezia* spp. to proliferate in a niche where lipid rich sebum is abundant (scalp and other seborrheic regions) in order to compensate their lipid dependence based on a dysregulated synthesis of myristic acid [11], which is compensated by the over-activity/expression of hydrolases for the production of medium-length or long-chain fatty acids [12]. Except *M. pachydermatis*, all *Malassezia* species require fatty acids to survive as their carbon source. Another characteristic of the cell wall of *Malassezia* is the Mala s 1 protein exposed on cell surface, being a major allergen in *Malassezia*-related disorders of the skin [13,14]. Recent studies demonstrated that *Malassezia* spp. could directly stimulate the production of inflammatory cytokines, chemokines and adhesion molecules in human epidermal keratinocytes [8,15]. Mishra *et al.* reported that this host immune activation is also characterized by the stimulation of T-cell reactivity and IgE production [16]. A peculiar aspect of *Malassezia* spp. is its reproduction occurring by monopolar or unipolar budding. The extruded bud is separated by a septum from the mother cell and successive scars form a small collar (collarette) (Fig. **1**) [12]. These features can differentiate *Malassezia* from other yeasts.

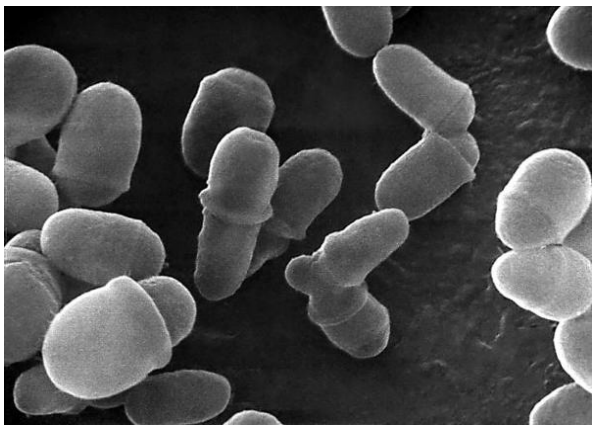


Fig. (1). Reproduction of *Malassezia* by budding.

Another class of important enzymes found in *M. furfur* is tryptophan aminotransferases, responsible of the conversion of L-tryptophan to different indolepyruvate metabolites [11]. Malassezin is a tryptophan metabolite and induces apoptosis in human melanocytes by means of a direct binding to the aryl hydrocarbon receptor (AhR) [17]. As other microorganisms, *M. furfur* is also able to form biofilm *in vitro* [18] and *in vivo* as protective mechanism for evasion of immune surveillance and as barrier against antimicrobial agents. Conversely, *M. globosa* and *M. restricta* secrete several lipases that can be categorized as Family Lipase 3 and Family LIP 2, both responsible of the production of fatty acids endowed with inflammatory effects [19-24].

The pathogenicity could be also correlated with host hormonal, metabolic or immunological disorders and predisposing environmental circumstances (temperature and humidity, and genetic susceptibility) that alter the cutaneous lipid profile. Nowadays, *Malassezia* spp. were shown to be the etiological agents of a large number of superficial skin diseases including pityriasis versicolor (PV) [25-27], *Malassezia* folliculitis [28], seborrheic dermatitis (SD) [29-31], dandruff [32-35], and atopic dermatitis (AD) [36-39]. Until recently, only *M. furfur* has been thought to be responsible for the onset of dandruff. However, the scalp specific species *M. globosa* and *M. restricta* have recently been found to be the most probable causative agents [40]. Finally, *Malassezia*-related fungaemia, associated to cardiac and pulmonary infections, usually occurs in young patients through the not correct use of catheters.

2. LITERATURE SURVEY FOR THE TREATMENT OF *MALASSEZIA* SPP.

Current treatment of dandruff/seborrheic dermatitis takes advantage of a limited arsenal of anti-fungal drugs such as ketoconazole (KTZ), coal tar, zinc pyrithione, piroctone olamine, triclosan, selenium sulfide and lipase inhibitors. In addition to azole derivatives, the treatment options for *M. pachydermatis* infection also include chlorhexidine (Fig. **2**). Shampoos and other cosmetic formulations (lotions and conditioners), containing active ingredients such as ketoconazole (1%), miconazole (2%), chlorhexidine (2-4%) or combinations of them, are commonly used for *Malassezia* dermatitis treatment. Disregarding its efficacy against *Malassezia*-related infections, ketoconazole is known to have low clinical safety.

As a standardized protocol to assess the antifungal efficacy against *Malassezia* spp. has not been disclosed by Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [3], the data should be considered with caution because the absence of clinical breakpoints. In this regard, the epidemiological cut-off values (ECVs) are important to discriminate between susceptible and resistant isolates. Further investigations and studies are crucial for correlating *in vitro*

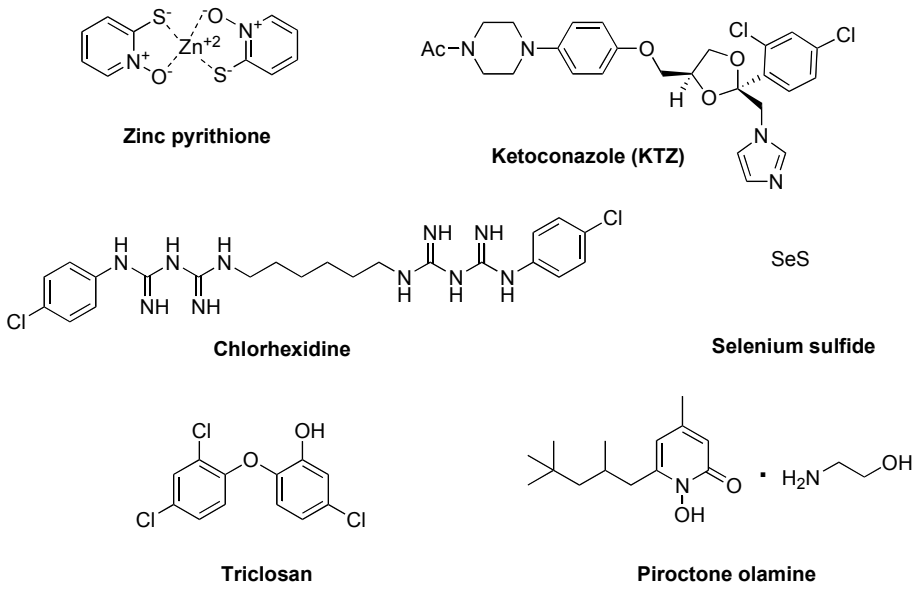


Fig. (2). Common arsenal for the treatment of *Malassezia*-related dandruff.

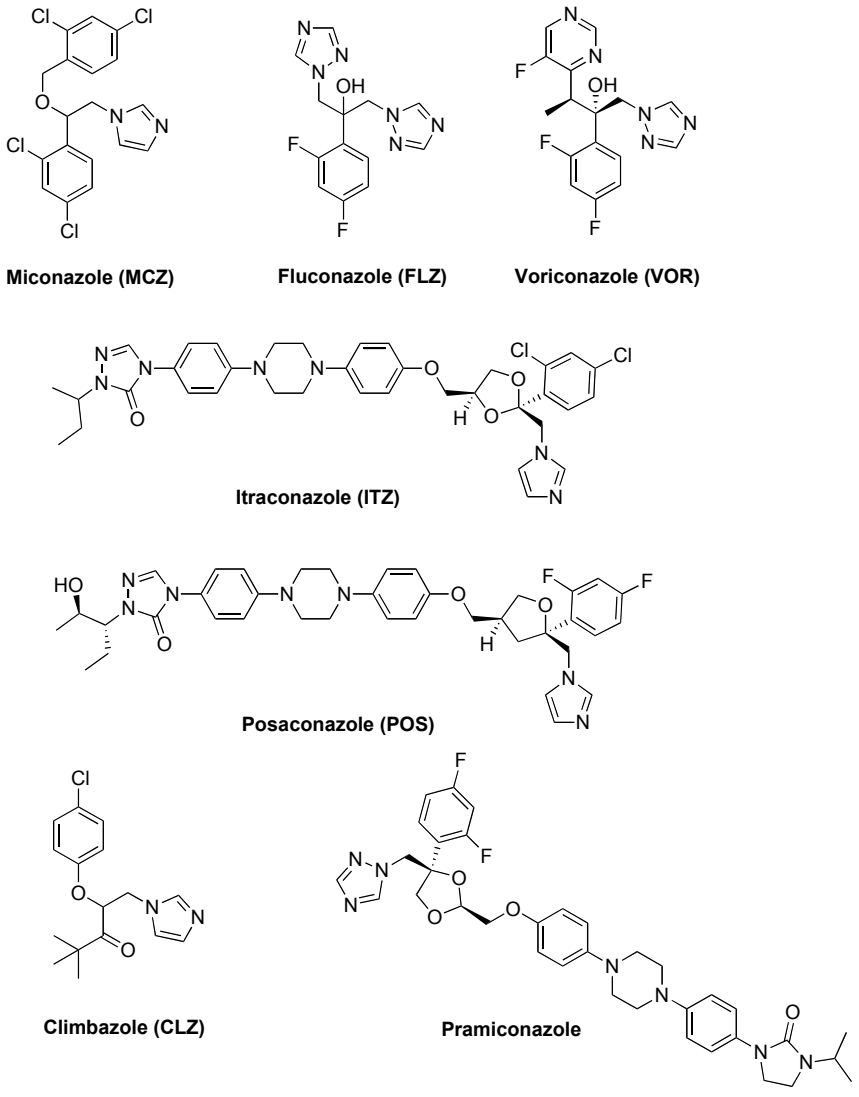


Fig. (3). Azole compounds tested against *Malassezia*.

Table 1. The most recent data about *Malassezia* spp. susceptibility to azoles, MIC values and characteristics of the isolated strains.

Strain (n° of isolates)	Source	Azole Drug	MIC^{a}_{50} (μg/mL)	MIC^{a}_{90} (μg/mL)	Further Information	Reference
M. globosa	human patients diagnosed with facial symmetric mild-to-moderate seborrheic dermatitis	CLZ	0.3125	-	4-week single-center, open-label split-face study	[47]
M. restricta		CLZ	2.5	-		
M. sympodialis		CLZ	0.3125	-		
M. slooffiae		CLZ	1.25	-		
M. pachydermatis (62)	dog skin	FLZ	8	32	epidemiological cut-off values were reported	[48]
		ITZ	0.008	0.016		
		POS	0.016	0.032		
		VOR	0.064	0.064		
M. furfur (60)	human blood and sterile sites	FLZ	64	128		
		ITZ	0.25	1		
		POS	0.25	0.5		
		VOR	1	2		
M. furfur (18)	human skin	FLZ	128	>128		
		ITZ	0.25	0.5		
		POS	0.125	0.25		
		VOR	2	2		
M. pachydermatis (72)	healthy dog skin	KTZ	0.094	0.125		[49]
		ITZ	0.047	0.119		
M. pachydermatis (110)	dog skin with atopic dermatitis	KTZ	0.33-1.33	1.6-5.2		
		ITZ	0.27-1.6	3-7.09		
M. furfur (39)	human patients with dermatological pathologies	FLZ	4	16		[50]
		ITZ	0.03	0.06		
		KTZ	0.03	0.06		
		VOR	0.06	0.25		
		MCZ	1	4		
M. sympodialis (20)	human patients with dermatological pathologies	FLZ	0.5	2		
		ITZ	0.03	0.06		
		KTZ	0.03	0.03		
		VOR	0.06	0.06		
		MCZ	0.25	4		
M. globosa (14)	human patients with dermatological pathologies	FLZ	0.5	2		
		ITZ	0.03	0.06		
		KTZ	0.03	0.03		
		VOR	0.03	0.125		
		MCZ	0.25	2		

(Table 1) contd….

Strain (n° of isolates)	Source	Azole Drug	MIC[a]$_{50}$ (μg/mL)	MIC[a]$_{90}$ (μg/mL)	Further Information	Reference
M. pachydermatis (30)	dogs with external otitis and dermatitis	FLZ	2	4	fluconazole-sensitive strains	[51]
		ITZ	0.125	0.5		
		KTZ	0.03	0.06		
		VOR	0.25	2		
M. pachydermatis (30)	dogs with external otitis and dermatitis	FLZ	64	128	fluconazole-resistant strains	
		ITZ	16	64		
		KTZ	16	32		
		VOR	32	64		

[a]MIC= Minimum Inhibitory Concentration

data with clinical outcomes since the current published values do not provide definitive conclusions.

2.1. Azoles and their Derivatives

Several past studies have demonstrated the sensibility/resistance of *Malassezia* spp. isolated from humans or animals to the treatment with azoles (Fig. **3**) [41-45]. Moreover, ketoconazole and pramiconazole were shown to reduce, in a dose dependent manner, the production of hyphae in *M. furfur* and *sympodialis* [46].

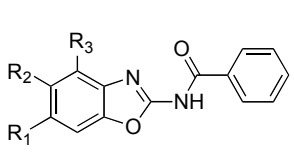
To gain new insights about the efficacy of this class of drugs and to establish putative epidemiological cut-off values (ECVs), several authors focused their efforts on testing clinical isolates of *Malassezia* spp. deriving from healthy (ecosystem inhabiting the skin) or compromised patients (humans or animals as reported in Table **1**). The Minimum Inhibitory Concentration (MIC) was defined as the lowest drug concentration without visible growth. MIC_{50} and MIC_{90} refer to MIC for 50 and 90 % of strains, respectively.

Collectively, azole drugs represent the first choice for an effective treatment of *Malassezia*-related infections. In detail, MICs for fluconazole were usually higher than those observed for other azoles, and it is not considered as a good choice for *Malassezia* treatment. Conversely, a wide range of MIC values was reported for miconazole, one of the most widely used topical drugs. The source of isolation was demonstrated to affect strain susceptibility because of the statistically significative differences registered in their MIC values.

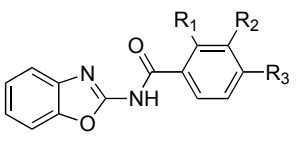
Surprisingly, the reported ECV values for azole drugs suggest that in *M. pachydermatis* and *M. furfur* different resistance mechanisms could be achieved, because *M. pachydermatis* displayed cross-resistance among the azoles, differing from *M. furfur*. The main defence mechanism has been shown to be the drug efflux pumps [52].

Pramiconazole is a broad-spectrum triazole antifungal (Fig. **3**) more active than ketoconazole against pathogenic *Candida* spp., dermatophytes, and 29 strains of *Malassezia* spp. (MIC <1 μg/mL) [53]. Moreover, it was tested orally for efficacy and tolerability in the treatment of seborrheic dermatitis [54] and pityriasis versicolor (randomized, multicenter, double-blind, placebo-controlled, 28-day, dose-finding study) [55]. A statistically significant dose-dependent effect was observed and there were no treatment-related adverse events up to 1 month after treatment onset.

Starting from the promising antifungal activity obtained with several 2-(substituted phenyl or benzyl) benzoxazoles, and on the basis of the chemical skeleton of malassezin, benzoxazole amides were synthesized and evaluated against *M. furfur* using the disc diffusion method as bioisosteres of azole drugs. Ketoconazole was chosen as reference drug (MIC value of 16 μg/mL) for comparison (Fig. **4**) [17].



1 $R_1 = R_2 = R_3 = H$
2 $R_1 = R_3 = H$, $R_2 = Cl$
3 $R_1 = R_3 = H$, $R_2 = NO_2$
4 $R_1 = R_3 = H$, $R_2 = OCH_3$
5 $R_1 = R_2 = H$, $R_3 = CH_3$
6 $R_1 = CH_3$, $R_2 = R_3 = H$
7 $R_1 = R_3 = H$, $R_2 = COOH$
8 $R_1 = CF_3$, $R_2 = R_3 = H$



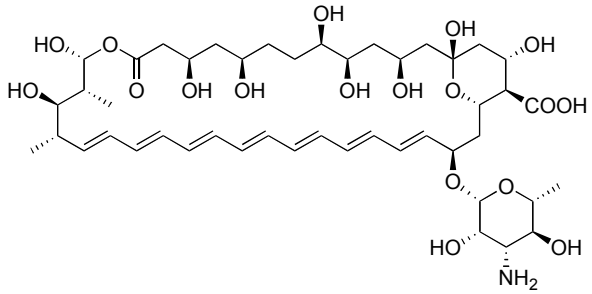
9 $R_1 = R_3 = H$, $R_2 = C_3H_6NS_2$
10 $R_1 = Cl$, $R_2 = H$, $R_3 = NO_2$
11 $R_1 = R_2 = H$, $R_3 = CH_3$
12 $R_1 = R_2 = H$, $R_3 = OCH_3$

Fig. (4). Benzoxazole amide derivatives.

Despite the substitution pattern, only few compounds displayed a slight inhibitory activity, and inferior to ketoconazole (the unsubstituted compound **1** with MIC value of 350 μg/mL). The 4-substitued analogs with nitro, methyl or methoxy groups (**10-12**) did not exert inhibitory activity, (MIC values >1000 μg/mL). In addition, they presented strong cytotoxic activity at 10 μg/mL against an immortalized human cell line (HepG2).

2.2. Amphotericin B

This well-established antifungal agent (Fig. **5**) has been proposed for the treatment of *Malassezia* spp. because it displayed low and promising MIC_{50} values against several clinical isolates of *M. furfur*, *sympodialis* and *globosa*, disregarding the experimental procedure used (broth microdilution and E-test), and the source of isolation [50, 56, 57].



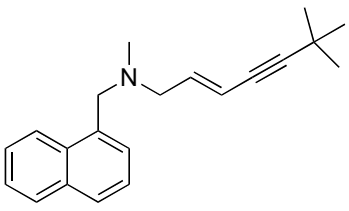
Amphotericin B

Fig. (5). Structure of Amphotericin B.

MIC_{50} values ranging from 0.125 to 0.5 μg/mL were also registered for this drug against *Malassezia* isolates considered as resistant by the M27-A3 document (like reported for *Candida* species), but no breakpoints for categorizing these yeasts as resistant to AMB were disclosed.

2.3. Terbinafine

Terbinafine (Fig. **6**) is a fungicidal drug belonging to the class of allylamines [42, 58].



Terbinafine

Fig. (6). Structure of terbinafine.

In order to study the susceptibility variations to this agent, 31 strains of *M. furfur, restricta*, and *globosa* were inhibited with MIC values spanning from <0.03 to 32.0 μg/mL, 0.06 to 4.0 μg/mL and 0.06 to 16.0 μg/mL, respectively. Susceptibility results of the *Malassezia* spp. for terbinafine were comparable to those obtained using LNA (Leeming-Notman agar medium). Only *M. sympodialis* isolates were generally susceptible to terbinafine (MIC≤0.25 μg/mL).

2.4. Selective *M. globosa* Carbonic Anhydrase (MgCA) Inhibitors (and Activators)

Carbonic anhydrases (CAs) are ubiquitous metalloenzymes extensively studied in bacteria, fungi, protozoa as well as in human tissues. Their physiological role involves vital and pathological processes [59] and depending on the structure and the type of the ion present in the active site they are usually divided into seven families (α-, β-, γ-, δ, ζ-, η-, and recently discovered θ-CAs), one of which (β class) has been well characterized in *Malassezia globosa* [60,61]. The presence of this versatile enzyme has been related to the uncontrolled growth and the induction of marked virulence in this fungus. Hence, the interference with the crucial activities regulated by CAs in microorganisms induces dysregulation of pH homeostasis, adenylyl cyclase activity, both sexual development and reproduction in filamentous ascomycetes as well as impairment of biosynthetic reactions mediated by acetyl-CoA carboxylase, pyruvate carboxylase, phosphoribosylaminoimidazole carboxylase and carbamoyl phosphate synthase, leading to significant antifungal effect both *in vivo* and *in vitro* [62]. Taking into consideration the emergence of resistant strains as a serious healthcare problem worldwide, the design of selective inhibitors of these fungal isoforms could open new scenarios for obtaining new anti-*Malassezia* agents endowed with a novel mechanism of action, since the β-CAs are not present in mammals (encoding only for α-CAs).

More in detail, *Malassezia globosa* has been recently studied for the presence of its specific β-CAs by Supuran's group [63,64]. This isozyme (MgCA) is endowed with outstanding enzymatic activity for the catalysis of CO_2 hydration and utilizes, in its long active site, one histidine, one aspartate (in the "closed active site"), and two cysteines for the coordination of the Zn(II), or one histidine, two cysteines, and a water molecule/hydroxide ion in the "opened active site".

A preliminary inhibition data analysis with inorganic/organic anions and other small Zn-coordinating molecules provided important structural information

Table 2. K_i **values against** *Malassezia globosa* **CA and MICs obtained with new inhibitors and clinically used compounds.**

Compound	K_i (nM) MgCA	MIC (μg/mL)			
		M. furfur CBS 9569	*M. dermatis* CBS 9145	*M. pachydermatis* CBS 6536	*M. globosa* CBS 7966
1	9800	640	640	>640	640
2	245	80	160	10	80
3	152				
4	6740	640	320	320	160
5	174	320	160	160	160
6	79	640	640	640	320
7	116				
8	123				
9	349				
10	543				
11	90	>640	>640	>640	320
12	92	640	640	640	640
13	79000				
14	85000				
15	236				
16	104				
17	63				
18	68				
19	35000				
20	234				
21	118				
22	94	>640	>640	640	640
23	4530	160	>640	160	320
24	2560				
25	3100				
26	650	320	320	320	320
27	374				
28	413				
29	660				
30	2750				
31	710				
32	220				
33	8090				
34	3490				
35	670				

(Table 2) contd….

Compound	K_i (nM) MgCA	MIC (μg/mL)			
		M. furfur CBS 9569	*M. dermatis* CBS 9145	*M. pachydermatis* CBS 6536	*M. globosa* CBS 7966
36	4500				
acetazolamide (AAZ)	76000	640	320	640	320
methazolamide (MZA)	74550				
ethoxzolamide (EZA)	38000				
dichlorphenamide (DCP)	346	>640	>640	>640	320
dorzolamide (DZA)	79000				
brinzolamide (BRZ)	84000				
benzolamide (BZA)	482				
topiramate (TPM)	1460				
sulpiride (SLP)	320				
indisulam (IND)	113				
zonisamide (ZNS)	7650				
celecoxib (CLX)	34800				
valdecoxib (VLX)	31500				

about the requirements for inhibiting this enzyme [65]. A large panel of anions (perchlorate, (seleno)cyanide, nitrate, halides, azide, carbonate, perrhenate, sulfate, nitrite, stannate, bisulfite, peroxydisulfate, selenate, perosmate, diphosphate, tellurate, hydrogen sulfide, divanadate, fluorosulfonate, hexafluorophosphate, triflate, tetraborate, trithiocarbonate, (thio)cyanate, and tetrafluoroborate), usually acting as weak inhibitors in other CAs, was not active against this fungal isoform (millimolar range). Among the most potent inhibitors (sulfamate, sulfamide, phenylarsonic and phenylboronic acids), bicarbonate (K_i = 590 μM) and diethyldithiocarbamate (K_i = 300 μM) were quite unexpected and comparable with the reference drug, acetazolamide, because the former is obviously also a substrate/reaction product of the CA-mediated physiological reaction. Conversely, Table **2** collects the MgCA inhibition data with a series of well known and clinically used hCA inhibitors (Fig. **7**) [63].

Other sulfonamide derivatives **1**−**36** (Fig. **8**) were also included in this study.

These following structure-activity relationships (SARs) can be detected:

(i) Many compounds, especially the heterocyclic sulfonamide derivatives, were endowed with a fair MgCA inhibition (K_is range = 2.56−76 μM);

(ii) Some of them displayed a very potent enzyme inhibition *in vitro* (K_is range = 104−650 nM). These comprehend 4-substituted benzenesulfonamides, halogenosulfanilamides, aminobenzolamides, as well as **DCP**, **BZA**, **SLP** and **IND**;

(iii) Few compounds (**6**, **11**, **12**, **17**, **18**, **22**), belonging to benzenesulfonamide or 1,3-disulfonamide scaffolds, had K_i in the range of 63-94 nM.

Compounds **29**-**36** were obtained by direct *N*-nitration of the sulfonamide group, trying to evaluate the impact on biological activity of a nitro substituent, that could improve the interaction with the Zn(II) in the active site reinforcing the acidity of SO_2NH moiety [66]. The fungal isoform was efficiently blocked by these *N*-nitro sulfonamides functionalized with small hydrophilic substituents at para position of the aryl ring, with K_is ranging between 0.22 and 8.09 μM. These results were better than those of the reference sulfonamide drug **AAZ** (K_i of 74.0 μM). Moreover, these *N*-nitro sulfonamides presented a selective inhibition of MgCA with respect to other human (hCA

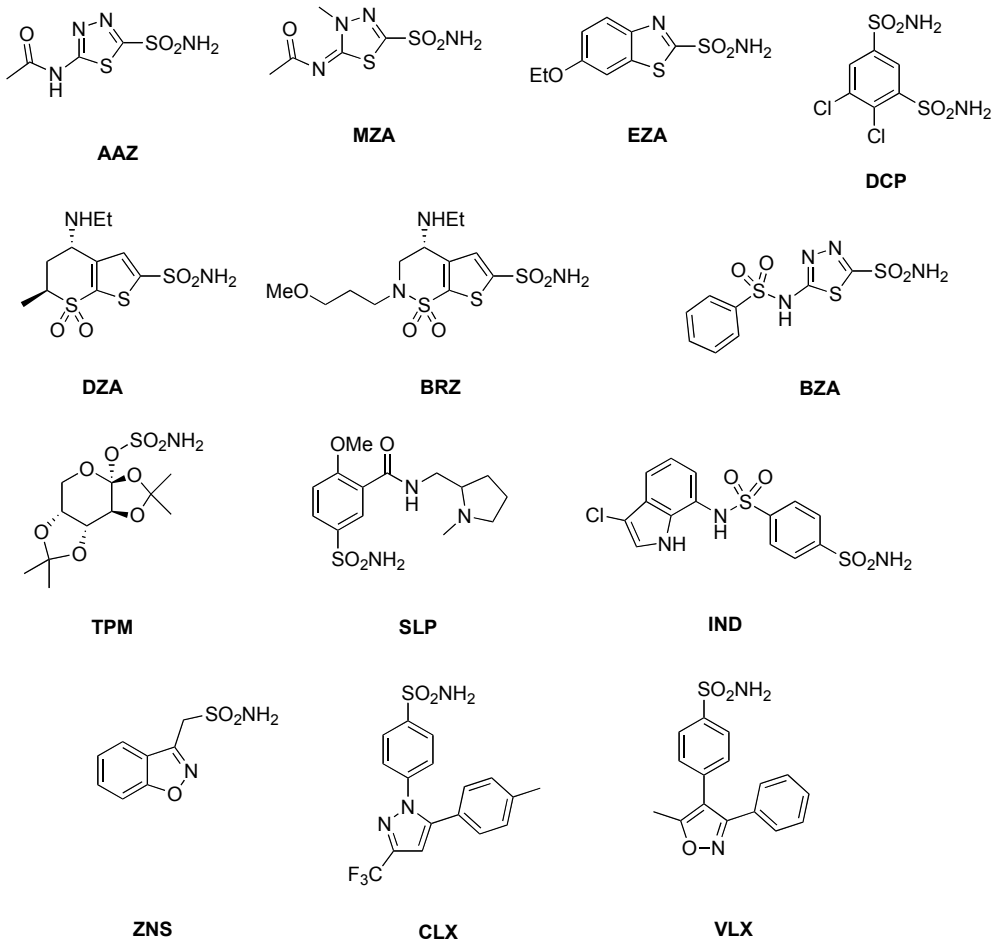


Fig. (7). Sulfonamide drugs tested as *Malassezia globosa* carbonic anhydrase inhibitors.

II) and pathogenic fungal β-CAs (high selectivity degree), and MIC values against four strains of *Malassezia* spp. *in vitro* for the most potent derivatives were also registered (Table **2**). Moreover, compound **6**, with K_i of 79 nM against MgCA *in vitro* and medium MIC values, was chosen for the treatment of a murine model of dandruff/*Malassezia* infection; 67% of the mice demonstrated clinical improvement. These results demonstrated that the inhibition of this enzyme (MgCA) could correlate with *in vitro* and *in vivo* treatment of *Malassezia* infections.

However, one of the main issues is that these inhibitors are functionalized with primary sulfonamides and their isosteres, usually recognized also as potent inhibitors of human CAs with the development of side effects [67-78]. For this reason, boronic acid derivatives (Fig. **9**) could behave as alternative chemotypes, as proposed in the very recent literature [79,80]. The peptidomimetic boronic acid bortezomib is clinically used for the therapy of haematological tumours.

Bortezomib inhibited promisingly both α- and β-class CAs in the low micromolar range [81]. The MgCA enzyme showed affinity for bortezomib with K_i of 3.24 μM. Phenylboronic acid (Fig. **9**), instead, behaved as rather a weak inhibitor of MgCA, with K_i of 89 μM. These data suggest that aliphatic boronic acids could be in detail explored in order to find new potent MgCA inhibitors.

Another promising scaffold of dithiocarbamates, obtained from primary and secondary amines, was recently proposed (Fig. **9**). They were more potent than the reference drug (**AAZ**) with inhibition constants ranging from 383 to 6235 nM. The aliphatic chain length influenced positively the biological activity. Unfortunately, these derivatives were also strong inhibitors of human CAs, because they established a direct and strong interaction with the zinc ion in the active site (a common structural feature in most of CAs), as reported by molecular modelling studies [82].

Conversely, following a computational approach performed to suggest new chemotypes for the selective interaction with this enzyme [83], some MgCA activators (a series of amines and amino acids **1-19**, Fig. **10**) were proposed to modulate the β-CA catalytic/ activation

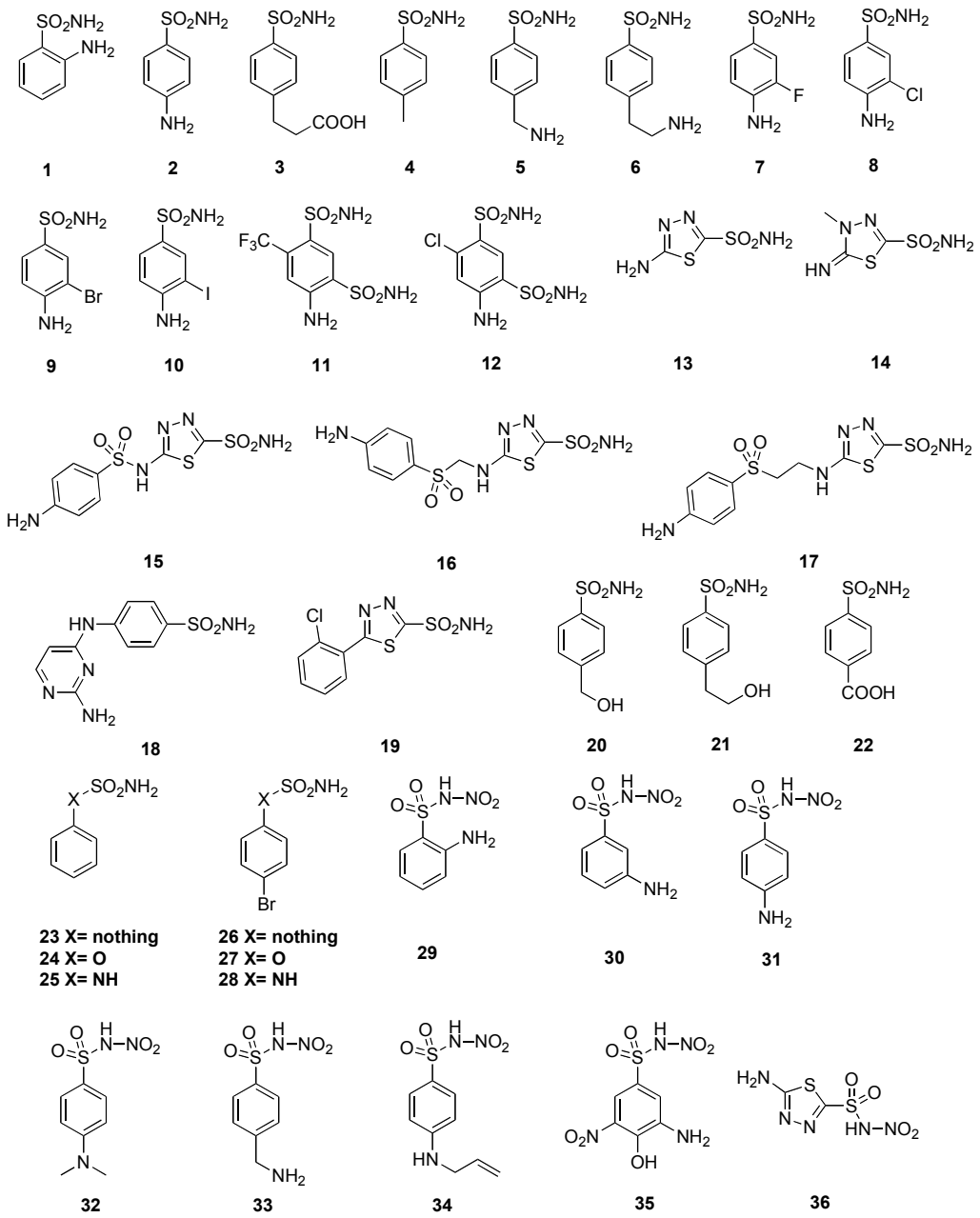


Fig. (8). Sulfonamide derivatives tested as *Malassezia globosa* carbonic anhydrase inhibitors.

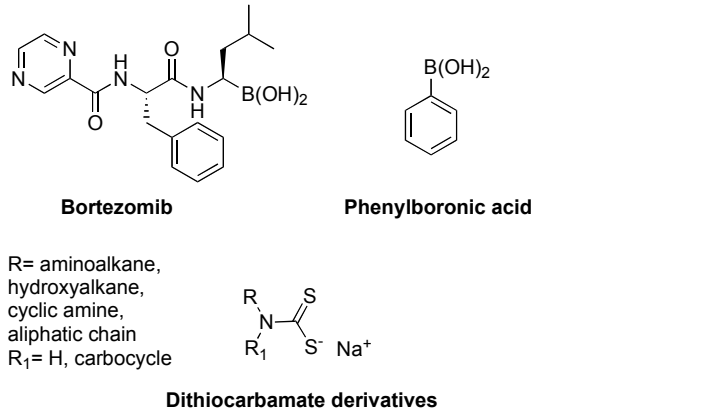


Fig. (9). Boronic acid derivatives and dithiocarbamates as innovative *Malassezia globosa* carbonic anhydrase inhibitors.

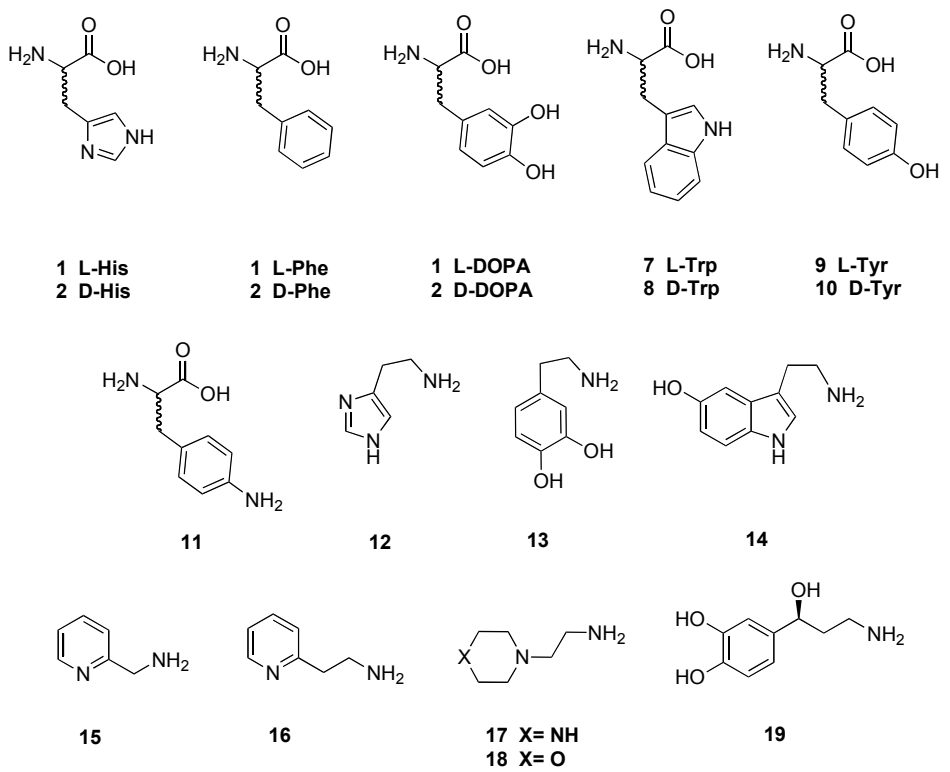


Fig. (10). Activators of *Malassezia globosa* carbonic anhydrase.

mechanism in *Malassezia globosa* [84]. Data showed that L-adrenaline **19** and 1-(2-aminoethyl)piperazine **17** were potent activators of MgCA, and that compounds bearing an amine group were generally more potent activators compared to those functionalized with a carboxylic acid moiety.

2.5. Bioactive Components and Extracts of Plants (2012-2017)

Natural products as source of promising agents against *Malassezia* have been partially reviewed in the past [85-87]. For this reason, in this paragraph, only papers published from 2012 were analyzed for an update on this matter. It emerged the role of specific phytocomponents such as saponins, xanthones, flavan-3-ols, and essential oils to display anti-*Malassezia* activity (Table **3**).

In addition, some authors screened *in silico* through molecular simulations two phytocomponents (β-Sitosterol and Calceolarioside A, Fig. **11**) showing a potent ability to bind to Mala s 1 [16].

2.6. Silver Nanoparticles

Drug-nanoparticle hybrid systems have widely been found useful in the enhancement of bioavailability, bioactivity and stability of clinically used drugs. Sulfonamides, sulfadiazine and sulfamerazine complexed with silver nanoparticles (AgNPs) as antifungal agents, showed enhanced activity against clinically relevant fungi. Moreover, these nanoparticles were able of limiting multidrug resistance and their formulations (antidandruff shampoos) were reported to act as effective against *M. furfur*-related dermal diseases.

AgNPs usually have a broadest spectrum fungicidal activity which makes them good candidates to eradicate fungal infection without recurrence. Moreover, silver is reported to enhance the generation of reactive oxygen species which in turn degrade the cell membrane and to catalyze the denaturation of S-S bridge in the cellular proteins. Several examples are reported in the literature both incorporating well known drugs or new chemical entities/natural substances.

The antidandruff activity of ketoconazole-coated silver nanoparticles by disc diffusion method was investigated against *M. furfur*. Antidandruff activity (MIC) was the highest with ketoconazole-coated AgNP (0.0135 mg/mL) when compared to ketoconazole alone (0.06 mg/mL) or AgNP alone (0.026 mg/mL). Moreover, they could act synergistically in combination being Ketoconazole active against fungal cell wall and AgNPs against intracellular targets. Thus, AgNPs not only act as a better antidandruff agent but could also reduce the side effects of ketoconazole by reducing its concentration [98].

Table 3. Natural products proposed and evaluated for the treatment of *Malassezia* spp.

Plant	Extraction Solvent	Antifungal Activity Against *Malassezia* spp.	Reference
Ricinus communis L. leaves	water, chloroform, methanol and petroleum ether	methanolic extracts exhibited significant activity (8.20 mm of inhibition zone), aqueous extracts recorded appreciable inhibitory activity (5.74 mm of inhibition zone) when compared with chloroform (1.66 mm of inhibition zone) and petroleum ether extracts (inactive) at 500 µg/mL concentration	[88]
Asparagus racemosus roots	fractioning extraction with *n*-hexane, 95% ethanol, distilled water, acetone, *n*-butanol to obtain different crude and saponin-enriched extracts	The inhibitory activity against *Malassezia globosa* and *furfur* of each extract was influenced positively by its saponin content, but it was always inferior to the reference drugs (zinc pyrithione and ketoconazole). No putative synergistic effects between *A. racemosus* extracts and zinc pyrithione or ketoconazole were demonstrated (FIC index <0.5 or >4)	[89]
Leaves of Evolvulus alsinoides, Azadirachta indica, Hibiscus rosa-sinensis, Lawsonia inermis, Murraya koenigii	ethanol	*Evolvulus alsinoides* exhibited MFC of 0.2 mg/mL (ZOI = 6 mm), whereas A*zadirachta indica, Lawsonia inermis* and *Murraya koenigii* displayed MFC values of 0.2 mg/mL (ZOI = 11-13 mm). On the contrary, *Hibiscus rosa-sinensis* exhibited lower fungicidal activity (1 mg/mL) and the lowest ZOI (2 mm)	[90]
H. perforatum roots	total methanolic extracts and fractions prepared with $CHCl_3$, EtOAc and MeOH	The MeOH extract, richer in xanthones, was the most active against *M. furfur* (MIC_{90} = 32 µg/mL). The inhibition % of biofilm formation, at concentration of 16 µg/mL, ranged from 14% to 39%; the best results were obtained with the $CHCl_3$ fraction.	[91]
Essential oils of T. kotschyanus, Z. multiflora, R. officinalis, A. sieberi, M. spicata, and H. persicum	water	*Z. multiflora* essential oil (rich in carvacrol) showed MIC_{90} values ranging from 30 to 80 mg/mL. *M. nana* isolates were the most susceptible (30 mg/mL), whereas *M. slooffiae* isolates were shown to be the least sensible to the treatment (80 mg/mL). Itraconazole (MIC_{90}: 2.8 mg/mL), amphotericin B (MIC_{90}: 3 mg/mL) and fluconazole (MIC_{90}: 7.8 mg/mL) were used as standard inhibitors	[92]
tea tree oil	water	A large number of *M. furfur* isolates were assayed providing MIC_{50} and MIC_{90} values of 0.25%.	[93]
C. aggregata lichen	ethanol	MIC (mg/mL) values of 2.72, 0.63, and 1.28 against *M. furfur*, *M. globosa* and *M. sympodialis*, respectively, while no activity was recorded against *M. restricta*. Fluconazole was used as the reference standard (MIC values ranging from 0.006 to 0.051 mg/mL)	[94]
Nyctanthes arbor-tristis L. leaves	ethanol	MIC values of the ethanolic extract for *M. globosa* 7966, *M. furfur* 1878, *M. restricta* 7877, and *M. sympodialis* 9974 ranged from 1.05 to 1.47 mg/mL (MFC= 3.12 mg/mL) and its effect influenced cell membrane integrity	[16]
Aloe barbadensis, *Hibiscus rosa sinensis*, *Lawsonia inermis*, *Snake guard*, *Wrightia tinctoria*, *Eucalyptus globulus*, *Azadirachta indica*, *Allium sativum*, *Allium cepa*, *Citrus limonis*, *Sapindus mukorossi*, *Trigonella foenum graecum*, *Emblica officinalis*, *Acacia concinna*	distilled water	ZOIs (cm) were evaluated by cup plate method. *Citrus limonis* and *Emblica officinalis* fruits displayed the best inhibitory activity (also in association)	[95]
Grape (*Vitis vinifera* L.) seeds	ethanol/water (7:3 *v*/*v*) acidified with formic acid at pH 3	The inhibitory activity has been correlated with the content of monomeric and polymeric flavan-3-ols (MIC_{50}= 32 µg/mL)	[96]
Malacalm (*Citrus aurantium* 1%, *Lavandula officinalis* 1%, *Origanum vulgare* 0.5%, *Origanum majorana* 0.5%, *Mentha piperita* 0.5%, *Helichrysum italicum* var. *italicum* 0.5%)	commercial product of a mixture of essential oils in sweet almond oil and coconut oil	MIC value for Malacalm was lower than single essential oils. The most abundant components were also tested *in vitro* against *M. pachydermatis* being thymol and carvacrol the most promising anti-fungal agents. *In vivo* tests were also performed	[97]

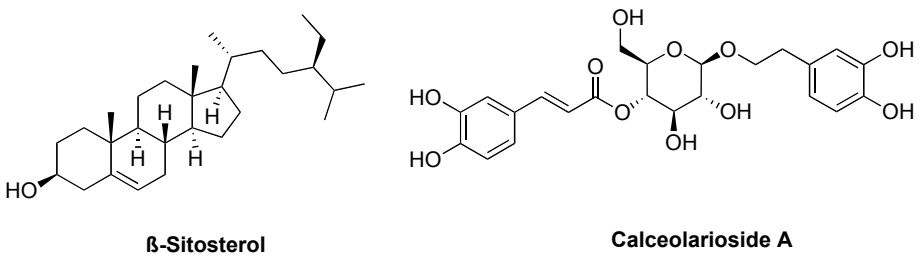


Fig. (11). Natural compounds able to interact with Mala s 1.

A large number of plant extracts (*Eucalyptus globulus*, *Aloe vera*, *Zingiber officinale*, *Wrightia tinctoria*, *Cassia alata*, *Azadirachta indica*, *Phyllanthus emblica*) were tested for their inhibitory activity against *M. furfur* (see previous paragraph). In cosmetic formulations there is a preference for natural surfactant (saponin)-based shampoos. Recent studies proposed natural saponins from *Sapindus mukorossi*, *Vernonia cinerea*, *Asparagus racemosus*, *Ricinus communis* and *Acacia concinna* as promising antimicrobial, antioxidant and antidandruff agents. The synthesis of AgNPs was obtained using the *Aegle marmelos* or *Acacia auriculiformis* aqueous extract [99]. The addition of AgNPs hampered the resistance of *M. furfur* 1374 to the treatment and limited the final dose suitable for the antifungal efficacy. The zone of inhibition (ZOI) towards *M. furfur* increased following the reduction of nanoparticle size. In addition the cell shape and surface were significantly altered after the treatment with AgNPs.

Antifungal activity of spherical- and rod-shaped AgNPs was investigated against *M. furfur* [100]. AgNPs and known antifungal drugs such as ketoconazole and itraconazole, as reference drugs, were challenged by agar diffusion method. It was determined that 20 nm NPs induced ZOI values higher than 50 nm spherical NPs. 20 nm spherical-shaped AgNPs at 0.5 mg/mL exhibited antifungal activity higher than 50 nm rod-shaped AgNPs. Smaller AgNPs were more promising for antifungal drug development owing to a deeper cell wall rupture, a reduced cellular respiration and cell death. The MICs were 0.2 mg/mL for 20 nm and 50 nm AgNPs and 0.3 mg/mL of rod-shaped nanoparticles. Successively, *in vivo* antimicrobial effect and tolerability of AgNPs on rat's skin surface were evaluated. On continued application of AgNPs, regression in redness was visible upon skin permeation. No pathological signs were found in any sacrificed animal. The activities of specific antioxidant enzymes were also preserved.

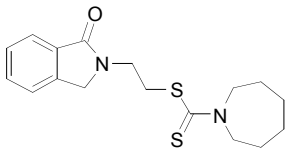
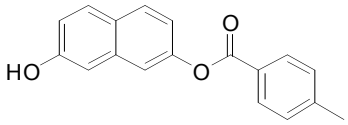
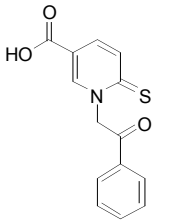
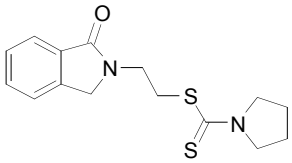
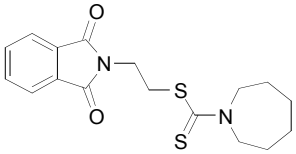
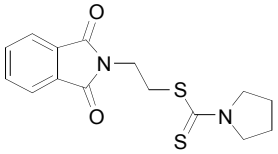
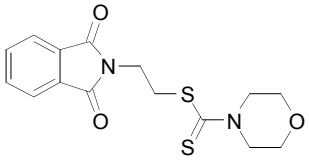
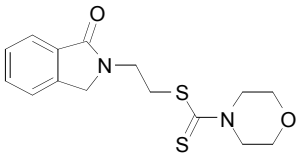
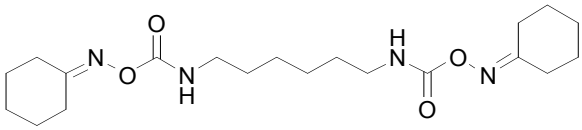
F. oxysporum, F. oxysporum f. sp. *vasinfectum* and *M. phaseolina* also showed the ability to reduce the aqueous silver ions into AgNPs and antifungal activity against *M. furfur* [101] AgNPs synthesized from *M. phaseolina* showed the maximum antifungal activity against *M. furfur* (24 mm of ZOI). AgNPs were also synthesised using flavonoids-enriched *Coriandrum sativum* leaf extract [102] and their *in vitro* anti-dandruff efficacy against *Malassezia furfur* MTCC 1374 was assessed (MIC of 25 μg/mL).

2.7. Mono and Diacylglycerol Lipases of *M. globosa*: LIP1 (SMG1) Inhibitors

Starting from the available crystal structure of SMG1 (PDB: 3UUE) [103], a virtual screening on a total of 170,000 compounds, docked into the substrate binding pocket, was performed selecting 147 commercially available compounds for experimental testing on SMG1 Lipase assay using different substrates and RHC 80267, a well established inhibitor of mammalian diacylglycerol lipases, as a reference drug [19]. Values are reported in Table **4** as IC_{50}, a measure of the effectiveness of these substances in inhibiting this enzyme. IC_{50} is defined as the drug concentration required for 50% inhibition *in vitro*.

Comparing these biological data to molecular modelling studies, it emerged the higher inhibitory activity of **1** (seven-membered ring, IC_{50} = 20.09 μM) and **4** (five-membered ring, IC_{50} = 22.86 μM) with respect to the reference drug (IC_{50} = 75.25 μM). Compound **1** was shown to establish H-bonds with Leu172 and Thr101 (pivotal residues also for RHC 80267), as well as hydrophobic interactions with two different pockets in the active site, thus orienting the sulfur atom towards His281 and Gln282. These residues are strongly conserved in many lipases within Family Lipase 3 secreted by *M. globosa*. The binding affinity was unfavourable both when oxygen atom is added to the cyclic lateral substituent (six-membered ring, **8**) and a further carbonyl function was present in the oxo-isoindoline nucleus (**5**-**7**), limiting the number of molecular interactions within the enzyme. The other compounds, **2** and **3**, are less active (IC_{50} of 81.75 μM and 98.34 μM, respectively).

Table 4. Experimentally determined IC_{50} of the reported SMG1 inhibitors.

Compound	Structure	IC_{50} μM (Substrate)
1		20.09 ± 7.00 (pNPA) 0.19 ± 0.06 (monoolein) 24.36 ± 4.64 (pNPC)
2		81.75 ± 20.69 (pNPA)
3		98.34 ± 19.21 (pNPA)
4		22.86 ± 4.92 (pNPA)
5		63.60 ± 10.31 (pNPA)
6		>200 (pNPA)
7		>200 (pNPA)
8		>200 (pNPA)
RHC 80267		75.25 ± 10.30 (pNPA)

IC_{50}s are expressed as mean ± SE using pNPA (*p*-nitrophenol acetate), monoolein and pNPC (*p*-nitrophenol octanoate) as substrates.

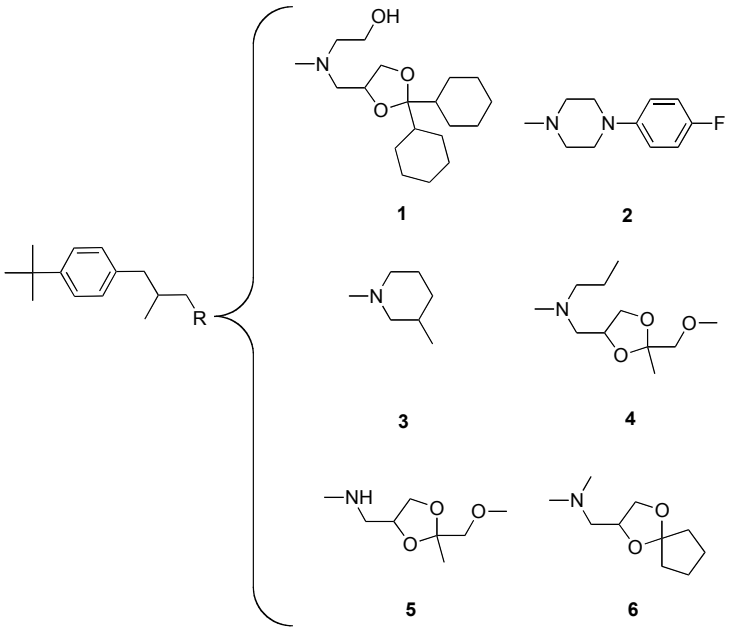


Fig. (12). Fenpropimorph derivatives.

To corroborate the biological activity of compound **1**, an additional lipase assay using monoolein, was carried out, showing a concentration-dependent inhibition with lower IC_{50}. It is also important to highlight that IC_{50} values of compound **1** did not change using other substrates (from 6.7 mM with pNPA to 41.7 μM with pNPC). Moreover, according to the Lineweaver-Burk plots, this inhibitor acted in a competitive fashion, as well as RHC 80267.

2.8. Fenpropimorph Derivatives

Fenpropimorph derivatives (**1**-**6**, Fig. **12**) were chosen not only as inhibitors of the growth of one strain of *M. furfur* (CCY 85-2-1) and two strains of *M. pachydermatis* (CCY 85-1-5, CCY 85-1-10) in comparison with bifonazol, but also for their ability to change the composition of the fungal membrane fatty acids, rather than their effects on ergosterol. The minimum inhibitory concentrations were determined by the microdilution method [104].

Compounds **1** and **2** were weak inhibitors for all tested strains, whereas **3** and **4** were only medium effective against two isolates of *M. pachydermatis*. Finally, compounds **5** and **6** strongly reduced the growth of *Malassezia*. Moreover, the presence of compound **5** induced adaptive changes not only in the sterols profile, but also in fatty acids content and composition.

2.9. Glutathione

Glutathione (GSH, Fig. **13**) is a sulfur-containing tripeptide, which neutralizes reactive oxygen species and their cytotoxicity. Moreover, this compound protects from pathogens, decreasing their virulence and enhancing their susceptibility to antimicrobial agents [105].

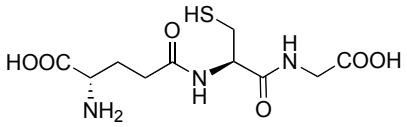


Fig. (13). Structure of glutathione.

This natural agent did not infer any inhibitory activity against GSH-treated *M. furfur* cultures ranging from 200 to 1000 μg/mL, when compared with untreated cultures, but it was shown to:

1. inhibit cell surface hydrophobicity (CSH) at 400 μg/mL (from 84% to 95% in four *Malassezia* spp.). CSH is a pivotal virulence factor contributing to form biofilm, to reduce phagocytosis of pathogens, and to enhance the release of inflammatory cytokines. CSH also strictly depends on the huge amount of lipids in *Malassezia* cell wall;
2. delay cell aggregation reducing CSH value without interfering with the cell surface charge, as demonstrated by the anti-hydrophobicity activity (AHA) of GSH and through FT-IR analysis;
3. modulate cell surface charge (CSC) in *M. furfur* as extrapolated by Zeta Potential measurements;
4. reduce Hydrophobicity Index (HI) at 400 μg/mL by evaluation of the minimal hydrophobicity inhibitory concentration (MHIC) value.

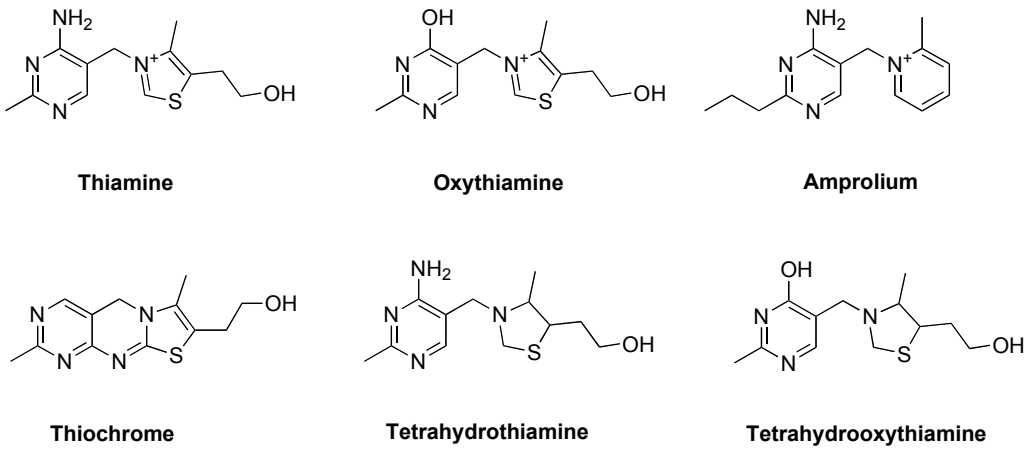


Fig. (14). Thiamine and its derivatives tested against *Malassezia*.

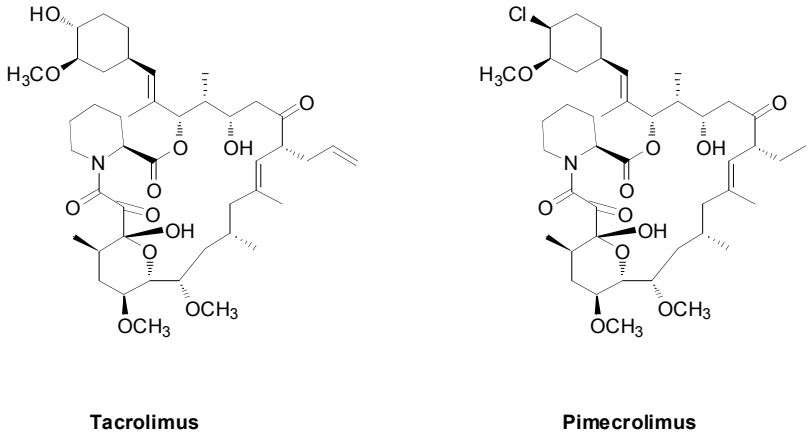


Fig. (15). Calcineurin phosphatase inhibitors.

2.10. Thiamine Derivatives

Taking into advantage of the cell growth inhibition in *S. cerevisiae* by thiamine analogs (Fig. **14**) responsible of impairment of important thiamine diphosphate-dependent enzymes activity, the Krebs cycle and pentophosphate pathway, a recent research investigated their antifungal effect against *M. pachydermatis* [106].

Only oxythiamine was the most promising as regards the growth inhibition at 40 μg/mL, probably due to its specific intracellular phosphorylation by pyrophosphokinase and its incorporation in thiamine-containing enzymes. Oxythiamine assimilation also causes pyruvate accumulation, inhibition of malate dehydrogenase, Krebs and glyoxalate cycles reducing energetic production and, eventually, the total fatty acids content of the cell wall of *M. pachydermatis*.

2.11. Pimecrolimus and Tacrolimus as Calcineurin Phosphatase Inhibitors

Pimecrolimus and tacrolimus (Fig. **15**), two ascomycin macrolactam derivatives, are calcineurin phosphatase inhibitors hampering both TH1 and TH2 cytokines release following T-cell activation and proliferation.

The former was tested against a large number of strains belonging to different *Malassezia* spp. (MICs ranging from 16 to 64 μg/mL) [41]. The latter also contrasted the *in vitro* growth of *Malassezia* spp. (MICs from 16 to 32 μg/mL) [107]. The rationale of this approach took advantage of the following evidence:

- a calcineurin homologue has been reported in fungi and tacrolimus also exerted a toxic effect against *C. neoformans* and *C. albicans* [108];
- from synergism test, when itraconazole and ketoconazole were added to tacrolimus, their corresponding MIC values were lowered. The FICI, calculated dividing the MIC of the combination of compound and the antifungal reference drug by the MIC of compound or antifungal reference drug alone, and interpreted as indicating a synergistic effect when it was ≤0.5, as additive or indifferent when it was >0.5 and ≤2, and as antagonistic when

it was >2) [109,110] was below 0.5 (synergistic effect) against *M. globosa*, *M. restricta*, *M. furfur* and *M. sympodialis*.

- the comparative efficacy of topical administration of tacrolimus with respect to clotrimazole has been also assessed in a single blind, randomized clinical trial for the treatment of pityriasis versicolor [111].

CONCLUSION

Due to the emerging number of *Malassezia* spp.-related infections over the last few decades, it is mandatory for the medicinal chemists to provide new and innovative tools to eradicate this lipophilic yeast or reduce its uncontrolled growth in predisposing conditions. The limited arsenal currently available for this treatment, which is mainly composed by old azole drugs, could not stem recurrence and cross-resistance. For this reason, we have collected novel and very recent approaches designing MgCA inhibitors, lipase inhibitors, AgNPs combined with natural products, calcineurin phosphatase inhibitors and other compounds for the treatment of *Malassezia* spp..

Despite *Malassezia* spp.-related infection are not always considered harmful or lethal, their occurrence in the general population (humans and animals) is increasing with long-lasting or relapsing phenomena according to the host conditions. Moreover, a direct interplay between fungal components and immune system has been demonstrated. Moreover, the antifungal susceptibility of *Malassezia* spp. to currently used drugs lacks of a standardized method and validated clinical breakpoints for assessing a proper treatment. Azoles and few other compounds are reported to be the first-line arsenal against *Malassezia* infections, disregarding several limitations, side effects and occurring resistance. In the absence of adequate guidelines by CLSI and EUCAST, there is an unmet need to identify new synthetic or natural compounds endowed with anti-*Malassezia* activity. In view of these premises, medicinal chemists focused their attention on alternative therapeutic targets (beyond the over-abused fungal 14α-demethylase) which are considered to be more specific of this microorganism.

Nowadays, *M. globosa* carbonic anhydrase (MgCA) inhibitors seem to be the most promising approach due to a better *in vitro* and *in vivo* evaluation of their efficacy in the collected literature. Not only the exact knowledge of the kinetics/structure of this enzyme, but also the increasing design of a plethora of inhibitors enlarged the available arsenal of new antifungal agents with limited toxicity. Conversely, lipase or calcineurin phosphatase inhibitors should also be another innovative field of research, but up to date few inhibitors were designed and tested to validate this approach. Other compounds were scarcely characterized in terms of antifungal activity (MIC, MFC, FICI), cytotoxicity against human cell lines, target selectivity and pharmacokinetics. However, the possibility of combining two drugs usually gave the best results as demonstrated for AgNPs encapsulating azole drugs or natural products. In addition, these nanomaterials could be efficiently compatible with several cosmetic formulations (e.g., shampoos, lotions and conditioners).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

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