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Synthesis and Biological Activity of New Antifungal Compounds: A Preliminary Study

Jorge A. Cabezas^{#*}, José A. Brenes[#], Norma Gross[^] and María L. Arias[^]

[#] School of Chemistry, University of Costa Rica, San José, 2060, Costa Rica

[^] Tropical Disease Research Center (CIET) and Faculty of Microbiology, University of Costa Rica, San José, 2060, Costa Rica

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Abstract

In the present study, we synthesized three 1,3-enynes from their corresponding aldehydes. They exhibited antifungal activity, which was evaluated against *Trichophyton rubrum*, the most common dermatophyte responsible for nail infection. The biological activity of these synthetic enynes was compared to terbinafine and ciclopirox olamine, two common antimycotic drugs used to treat nail infections caused by this pathogen. Two of these compounds showed an inhibitory effect similar to terbinafine and ciclopirox olamine, when tested against over eleven different *T. rubrum* strains, including ATCC 28188 strain and ten different clinical isolates, two already classified as multitreated.

Keywords: 1,3-enynes, 1,3-dilithiopropyne, propargylation, antifungal, onychomycosis, *Trichophyton rubrum*

1. Introduction

Onychomycosis is defined as a fungal infection of the nail. It affects toenails and fingernails, however it is known that toenails are 4 to 10 times more prone to infections than fingernails [1]. It occurs in about 10% of the adult population [2] and up to 90% of the older adults may be affected. Typically, these infections are characterized by white-yellow-brown discoloration of the nail, thicker and chalkiness nail plate and hyperkeratosis of nail bed.

The pathogens causing these infections include three types of fungi: dermatophytes, yeast and non-dermatophytic molds. More than 90% of toenail infections and more than 50% of fingernail infections are caused by dermatophytes [1],[3].

Dermatophytes represent a specialized group of fungi, commonly associated with cutaneous disease both for humans and animals [4]. They are keratinophilic microorganisms, capable of infecting superficial keratinized tissues, including skin, hair and nails [5].

The most common dermatophytes responsible for nail infection are *Trichophyton rubrum* and *Trichophyton mentagrophytes* [3]. Nevertheless some yeast like

Candida albicans, *Candida parapsilosis* and some non-dermatophytes moulds, such as *Fusarium*, *Neoscytalidium*, *Scopulariopsis* and *Aspergillus* are primary pathogens in some cases of fingernail onychomycosis [6]. In recent years, the number of infections caused by this group of fungi has increased significantly, representing a risk especially for immune-compromised patients, where severe and atypical manifestations may occur [7]. Also, the emergence of drug resistant forms has become a topic of concern [8].

Many antimycotic drugs have been developed during the past 40 years to treat fungal infections. Due to the slow-grow of nails, treatment of onychomycosis generally involves long-term therapy. Normally it consists of topical ointments applied directly to the nail, in cases of mild infections. This topical therapy has limited success due to poor permeability of applied drugs through the nail [9]. In cases of more severe infections, classic systemic drugs such as griseofulvin, **1**, and ketoconazole, **2**, have been used for a long time. These drugs must be used for long periods of time (10-18 months) and the major side effects of these drugs are liver toxicity and gastrointestinal and systemic side effects. Their relapse rate is 50-85%.

Griseofulvin, **1**, was first isolated in 1939 [10] from filamentous fungi *Penicillium griseo-fulvum*, and has been used as antifungal drug for decades. Some of its side

*Corresponding author's ORCID ID: 0000-0002-7571-6914

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effects include nausea, diarrhea and also has some teratogenic effects when used before pregnancy. Nowadays, this antifungal is not recommended to treat nail infections.

Ketoconazole, **2**, an imidazole class compound, discovered in 1976 at Janssen Pharmaceuticals, was introduced in U.S.A. in 1977 (Nizoral®). However, because it can cause serious liver injury, in 2013 the European Medicines Agency recommended to ban it for systemic use in the European Union [11]. In U.S.A. has been replaced by the triazoles, itraconazole, **3** and fluconazole, **4**. Oral antifungal treatments with itraconazole, **3** and fluconazole, **4** are taken for at least 12 months and have better cure rates and fewer side effects than Ketoconazole, **2**. However, they are associated with a marked increased risk of clinical acute liver injury [12]. Azole drugs inhibit the enzyme 14- α -lanosterol demethylase in ergosterol biosynthesis (the main fungal sterol), resulting in depletion of ergosterol and accumulation of toxic 14- α -methylated sterols in membranes of susceptible fungal species, having a fungistatic action.

The synthetic antifungal agent ciclopirox olamine, **5**, unrelated with imidazole derivatives, was approved by the FDA in 2006, and has been used in lacquers for treatment of superficial onychomycosis due to *Trichophyton rubrum* and *Trichophyton mentagrophytes*. In open and placebo-controlled studies, in patients with superficial dermatophyte or yeast infections, topical ciclopirox olamine, **5**, was comparable to or better than clotrimazole, **6**, in efficacy [13].

Terbinafine, **7**, was discovered in 1984 [14], and it was found to have higher activity than drugs such as ketoconazole, **2**, griseofulvin, **1**, econazole, **8**, and tolnaftate, **9**, in oral and topical treatment of guinea pig dermatophytoses [15]. Terbinafine, **7** has been used orally, to treat superficial toenail and fingernail infections, since 1996. It has the advantage that it concentrates in keratin-rich tissues, and it has a fungicidal effect [16]. It has been reported that it is less toxic to the liver than griseofulvin, **1**, and ketoconazole, **2** [17]. However when given orally, adverse effects such as hepatocellular necrosis and cholestatic injury has been reported [18]. The period of time between the start of medication and the development of the liver injury is about 4-6 weeks [12].

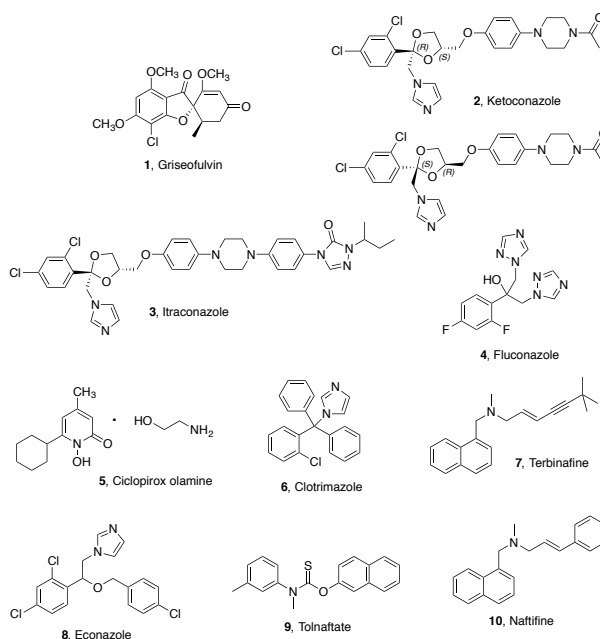


Figure 1 Example of some compounds with antifungal activities

It is thought that terbinafine, **7**, like other allylamines such as naftifine, **10**, binds to fungal squalene epoxidase, inhibiting ergosterol synthesis from squalene. This results in a deficiency of ergosterol changing its permeability and causing fungal cell lysis. Also this provokes an increase of the intracellular concentration of squalene, ultimately disrupting and damaging the cell wall, resulting in a fungicidal action [19].

The first antifungal compound of the allylamine-type was naftifine, **10**, [(*E*)-*N*-methyl-*N*-(1-naphthylmethyl)-3-phenylallylamine]. Some structure-activity studies, based on naftifine, **10**, lead to the discovery of the more active compound terbinafine, **7**, first pharmaceutical agent to contain a (*E*)-1,3-enyne in its chemical structure [20].

The development of different and novel antifungal drugs has been a primary objective for the scientific community. These innovative drugs must have different modes of action compared with actual antifungals, so that no cross-resistance appears. Nevertheless few marketed antifungal drugs have been introduced lately [21]. In the present study, a terbinafine-derivative **11**, and two new highly active 1,3-enynes **12** and **13**, were thus evaluated. These compounds were prepared by a synthetic methodology we developed, that allowed us to convert aldehydes and ketones into 1,3-enynes in a one-pot reaction [22]. The antifungal activity of these synthetic derivatives against *Trichophyton rubrum* was measured, and compared to terbinafine **7** and ciclopirox olamine **5**.

2. Results and Discussion

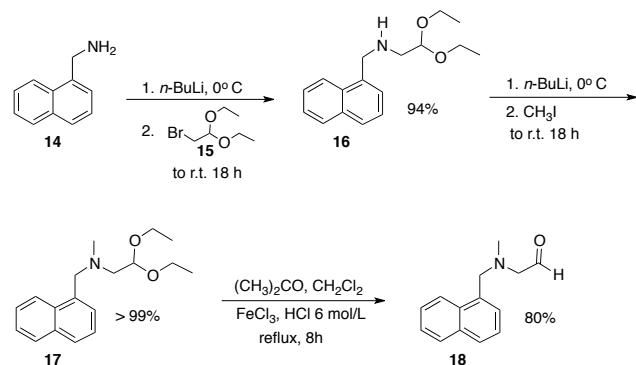
2.1. Synthesis

Antifungal agents such as naftifine, **10**, and terbinafine, **7**, are classified as allyl amine-type. It has been found that

the tertiary (*E*) allyl amine unit is essential for its biological activity [23]. Furthermore, two lipophilic groups (*tert*-butyl and naphthalene) linked by a polar spacer of a proper length, are needed for the biological activity of terbinafine, **7** [24].

We decided to use a recently developed procedure [22], for the one-pot conversion of aldehydes and ketones into 1,3-enynes, to prepare three compounds containing this enyne framework (**11**, **12**, and **13**), and to measure its activity against *Trichophyton rubrum*.

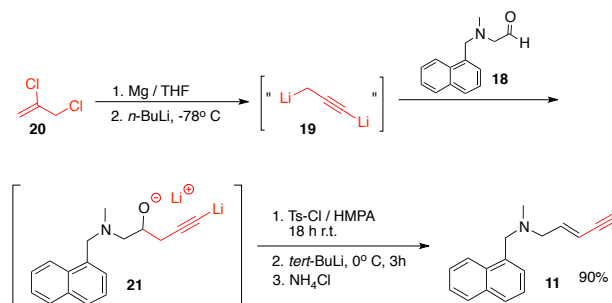
Enyne **11**, was prepared from aldehyde **18**. The synthesis of **18**, initiated with reaction of 1-naphthylmethylamine, **14**, with *n*-BuLi at 0°C, followed by treatment with bromoacetaldehyde diethyl acetal, **15**, to obtain the corresponding amino ketal **16**, in 94% yield (Scheme 1). The amine **16** was treated with *n*-BuLi at 0°C, followed by reaction with methyl iodide to obtain, after stirring overnight, the methylated derivative, **17**, in quantitative yield (Scheme 1). To de-protect the ketal group many methods [25] were unsuccessfully used. Refluxing compound **17**, in glacial HOAc generated aldehyde **18**, which rapidly decomposed in the acidic media as it was formed, as corroborated by GC-MS analyses of aliquots taken from the reaction mixture. Refluxing ketal **17**, for 8 hours, with acetone in the presence of FeCl₃ also failed to produce aldehyde **18**. In this case, it was thought that the amine, present in **17**, could be coordinating strongly with Lewis acid FeCl₃, deactivating its catalytic function. Thus, we repeated this reaction but adding a few drops of HCl 6 mol/L, to form the corresponding ammonium chloride salt of amine **17**. In this case, the desired aldehyde **18**, was obtained in 80% yield (calculated by GC).



Scheme 1 Synthesis of aldehyde **18**

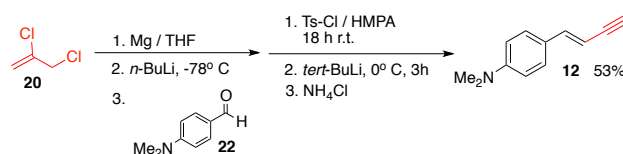
Several attempts to isolate aldehyde **18**, by column chromatography, resulted in decomposition of the product. This synthesis was repeated, and the crude reaction, containing this aldehyde, **18**, was taken into the next reaction step, without any further purification. Thus, we reacted aldehyde, **18**, with dianion "1,3-dilithiopropyne", **19**, which was prepared by treatment of 2,3-dichloropropene, **20**, with magnesium, followed by reaction with *n*-BuLi (Scheme 2) [22], [26]. The

intermediate, **21**, formed by addition of **19**, to **18**, was reacted with tosyl chloride, followed by elimination with *tert*-BuLi to afford, after aqueous work-up and column chromatography, enyne, **11**, in 90% yield (Scheme 2).



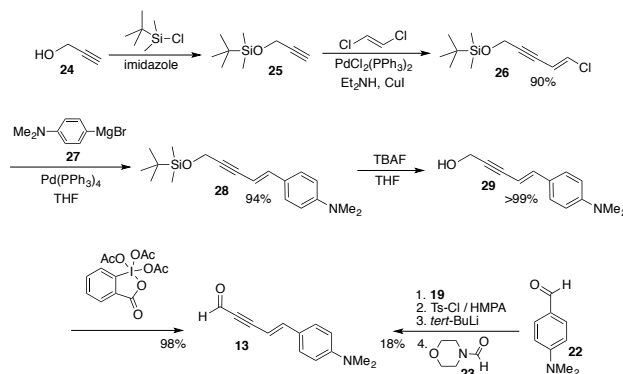
Scheme 2 Synthesis of enyne **11**

1,3-Enyne, **12**, was prepared by reaction of 1,3-dilithiopropyne, **19**, with *p*-dimethylaminobenzaldehyde, **22**, as previously described. This reaction gave product **12**, as a *trans:cis* isomeric mixture in a ratio of 9:1, in 53% yield (Scheme 3).



Scheme 3 Synthesis of enyne **12**

We decided to add a formyl group to the triple bond of enyne, **12**, to extend conjugation and possibly reactivity. Thus, we repeated reaction between *p*-dimethylaminobenzaldehyde, **22**, with dianion, **19**, and the reaction was quenched by addition of 4-formylmorpholine, **23**, to obtain, after aqueous work-up and column chromatography, aldehyde **13** in 18% overall yield (Scheme 4). Because this formylation process gave very poor yields, we decided to prepare aldehyde **13** by an alternative route.



Scheme 4 Synthesis of enyne **13**

Protection [27] of propargyl alcohol, **24**, gave the corresponding silyl ether, **25**, which was treated with *trans*-dichloroethylene, under Sonogashira reaction conditions, to obtain chloro enyne, **26**, in 90% yield. Palladium cross-coupling chemistry of this vinyl chloride, **26**, with 4-dimethylamino phenylmagnesium bromide, **27**, afforded enyne, **28**, in 94 %, which upon treatment with tetrabutylammonium fluoride gave alcohol, **29** in quantitative yield. Dess-Martin oxidation of alcohol, **29**, afforded desired aldehyde **13** in 98% and 82% overall yield (Scheme 4).

2.2 Antifungal Activity

The inhibitory effect of compounds **12**, **13** and terbinafine, **7**, was tested over a 1.0×10^4 spores /mL concentration of *T. rubrum*. Compound **12** showed a very weak activity, which disappeared at a concentration of 1,25 mg/mL, while compound **13** showed a behavior similar to terbinafine, **7**. This is shown on Figure 2, where the minimal inhibitory concentration obtained for compound **13** and terbinafine, **7**, was of 4g/ml.

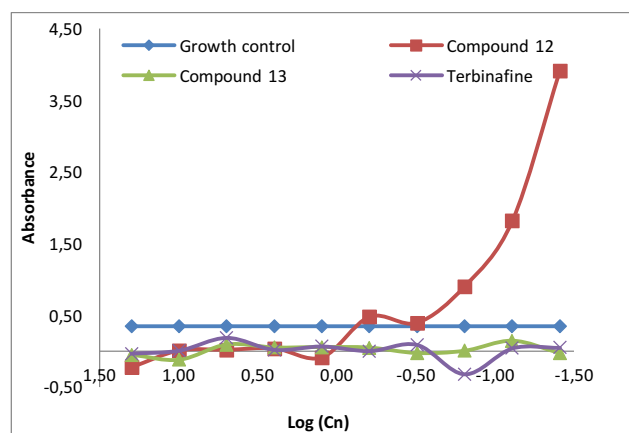


Figure 2. Inhibitory effect of compounds **12**, **13** and terbinafine, **7**, over a 1.0×10^4 spores /mL concentration of *T. rubrum*.

The inhibitory effect of compound **13** and terbinafine, **7**, over a 1.0×10^7 spores concentration of *T. rubrum*, was then tested. Both compounds showed a notorious, and similar, inhibitory effect over this dermatophyte (Figure 3).

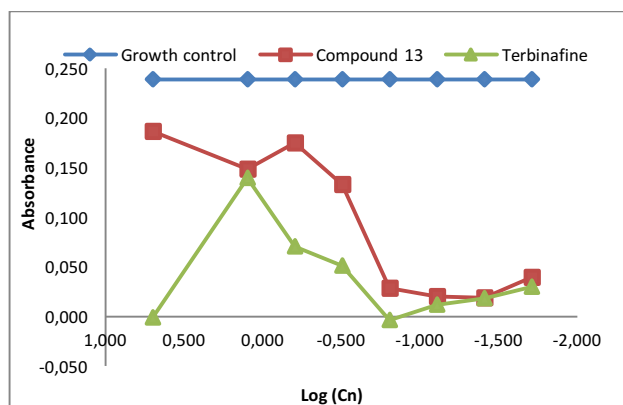


Figure 3. Inhibitory effect of compound **13** and terbinafine, **7**, over a 1.0×10^7 spores/mL concentration of spores of *T. rubrum*

The inhibitory effect of compounds **12**, **13**, terbinafine, **7**, and ciclopirox olamine, **5**, over eleven different *T. rubrum* strains, was determined. This study included ATCC 28188 strain and ten different clinical isolates, two already classified as multitreated (9 and 10), the comparison of these results is shown on Table 1.

Table 1. Comparison of inhibitory effect of compounds **12**, **13**, terbinafine, **7**, and ciclopirox, **5**, over *T. rubrum* strains

Mold	Positive Control	Compound 12	Ciclopirox 5	Compound 13	Terbinafine 7
1	+	+	+	+	+
2	+	+	-	-	+
3	+	+	-	-	-
4	+	+	-	-	-
5	+	+	-	-	-
6	+	+	-	-	-
7	+	+	-	-	-
8	+	+	-	-	-
9 ^a	+	+	-	+	-
10 ^a	+	+	+	+	+
11 (ATCC)	+	+	-	-	-

^a Multitreated. ^b (+) growth, (-) inhibition. ^c All trials were performed 4 times

Compound **12** does not inhibit any of the strains tested, but compound **13** inhibits 73% of the strains, a behavior similar to terbinafine, **7**. Only ciclopirox, **5**, inhibited 88% of strains. There is no statistical difference between terbinafine, **7**, ciclopirox, **5**, and compound **13** on the growth inhibition of *T. rubrum* ($p > 0,05$).

In addition, the inhibitory effect of synthetic derivative, **11**, was tested over a 1.0×10^4 spores /mL concentration of *T. rubrum*, and these results were compared to terbinafine, **7**, (Figure 4). This result indicates that compound **11** exhibit an excellent *in vitro*

inhibitory effect against *T. rubrum*; this inhibition is as good as the one shown by terbinafine, **7**.

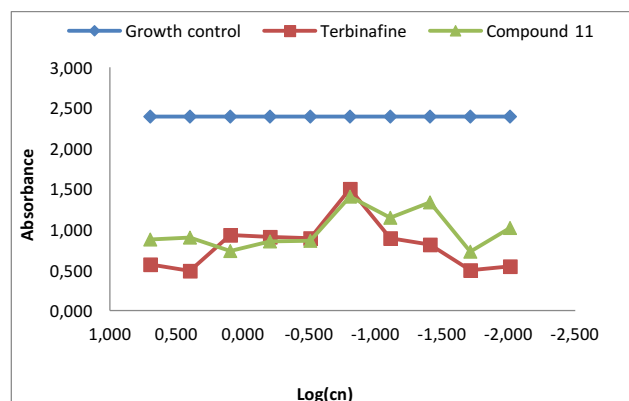


Figure 4. Inhibitory effect of compound **11** and terbinafine over a 1.0×10^4 spores/mL concentration of *T. rubrum*

Several categories of antifungal agents have been introduced into the market and routinely used, including itraconazole, **3**, fluconazole, **4**, and terbinafine, **7**. Nevertheless, there are several communications of bad therapeutic outcomes worldwide [28], of interactions with administrated drugs, which results in adverse clinical consequences [29] as well of an increasing development of resistance against them [30]. This situation has led to the development of several studies of the *in vitro* susceptibility of dermatophytes to new antifungal drugs, and the results have shown important variations, which may be due to methodological differences.

Although there are several antifungal agents very active against dermatophytes, it is important to point out that results obtained are species dependent and the development of resistance is probable, especially with dermatophytosis associated to *T. rubrum* [8].

3. Conclusions

In this study we developed one-pot syntheses, for compounds **11**, **12** and **13** (Schemes 1-4). Compound **13**, was also synthesized by an alternative and longer route, but with better yields (Scheme 4).

Compounds **12**, **13**, were evaluated over a 1.0×10^4 and 1.0×10^7 spores/mL concentration of *T. rubrum* and its biological activity compared to terbinafine, **7**. From these trials it was found that compound **13**, showed a similar inhibitory effect than terbinafine, **7**.

Compound **13**, also showed a similar inhibitory effect than terbinafine, **7**, and ciclopirox olamine, **7**, when tested against over eleven different *T. rubrum* strains, including ATCC 28188 strain and ten different clinical isolates, two already classified as multitreated. In these cases there were no statistical differences between terbinafine, **7**, ciclopirox, **5**, and compound **13** over the growth inhibition of *T. rubrum* ($p > 0.05$).

Additionally, the comparison of inhibitory effects of synthetic derivative, **11**, and terbinafine, **7**, over a 1.0×10^4 spores/mL concentration of *T. rubrum*, indicated that compound **11** has, *in vitro*, an inhibitory effect as good as the one shown by terbinafine, **7**.

4. Experimental section

4.1. Microbial trials

4.1.1. Test isolates

A total of 11 *Trichophyton rubrum* strains, including 10 clinical dermatophyte isolates and ATCC28188 were tested. All microorganisms are part of the Dermatophyte collection of the Medical Mycology Laboratory, University of Costa Rica.

4.1.2. Preparation of the fungal inoculum

T. rubrum was grown for 7 days at 35°C in test tubes containing Oatmeal agar. Spores were harvested by flooding test tubes with 85% sterile saline (1 ml), and lightly scraping the agar surface with a cotton swab. The spores were then counted in a Bürker hemocytometer and diluted in RPMI 1640 Medium® (Gibco™, Waltham, MA, USA) to a concentration of 1.5×10^4 and 1.5×10^7 spores/mL for tests of minimal inhibitory concentration determinations and to a concentration of 1.5×10^7 spores/mL for growth inhibition tests.

4.1.3. Antifungal tested and dilutions

Compounds A and D, as well as terbinafine (Royal Pharma) and ciclopirox (Jinlan Prham Drugs) standards were prepared at a 10 mg/mL concentration. Ten double dilutions were prepared using DMSO (dimethyl sulfoxide) for each product.

4.1.4. Comparison of the Minimal Inhibitory Concentration (MIC) of products A and D against terbinafine

Microtiter plates were inoculated with 100 µL of RPMI 1640 media, 100 µL of the *T. rubrum* spores and 100 µL of either products A, D or terbinafine dilutions. Growth and sterility controls were included for each test (growth controls consisted of spores, DMSO and RPMI 1640 media, sterility controls of RPM1 medium and DMSO only). Each trial was done by duplicate. Inoculated plates were incubated at 28°C for 4 days.

MIC was determined using Biotek Synergy HT multidetection reader (Vermont, US). Protocol followed included 450 nm lectures in order to determine the absorbance. The optic density of growth control wells were assumed as 100% growth. According to the microdilution reference method CLSI M38-A2 (Clinical Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of

filamentous fungi. Approved standard M38-A2. 2nd edition Wayne, PA: Clinical and Laboratory Standards Institute, 2008), final point was determined as 80% inhibition of this value, thus MIC was determined as the concentration of the last well where the optic density was 20% less of growth control.

4.1.5. Comparison of the efficacy on products A, D, terbinafine and ciclopirox in the growth inhibition of different *T. rubrum* samples.

For this test, 11 different isolates of *T. rubrum*, including ATCC 28188 and prepared as described above were evaluated.

Sabouraud dextrose agar plates with a final concentration of 20 µg/mL of either compound A, D, terbinafine or ciclopirox were inoculated in four different spots with 50 µL of a 1.0×10^7 *T. rubrum* spores/mL of each sample. Agar plates were incubated at 28 °C and in humid chamber for 10 days, in order to see the mold classical growth. Each sample was analyzed three different times.

4.2. Chemistry

General: All glassware and syringes were dried in an oven overnight at 140° C and flushed with nitrogen immediately prior to use. Transfers of reagents were performed with syringes equipped with stainless-steel needles. All reactions were carried out under a positive pressure of nitrogen. Nitrogen was passed through a Drierite gas-drying unit prior to use. Diethyl ether and tetrahydrofuran were refluxed and freshly distilled from sodium and potassium/benzophenone ketyl respectively, under nitrogen atmosphere. Hexane was distilled from sodium and collected and kept over activated molecular sieves. *n*-Butyllithium was titrated according to the method of Watson and Eastham [31]. ¹H-NMR and ¹³C-NMR spectra were recorded on a 400 MHz Bruker spectrometer. Low-resolution mass spectra were obtained on Agilent Technologies 7820A GC coupled to a mass spectrometer 5977E unit using electron impact at 70 eV. High resolution mass were measured on a Waters Synapt HMDS G1, Q-TOF. Infrared spectra were recorded on a Perkin Elmer FT-IR Spectrum 1000.

4.3. General procedure for the preparation of 1, 3-dilithiopropyne **19**:

An oven-dried, 100 mL, three-necked, round-bottomed flask was equipped with a magnetic stirring bar and a Liebig condenser bearing a glycerin-bubbler at the top. The exit of the bubbler, bearing a septum, was punctured with a double-tipped needle, whose other end was inserted, through a rubber septum, into a two-necked flask equipped with a magnetic stirring bar. The other neck of this flask was capped by a rubber septum bearing a needle attached to a balloon. All joints were greased

and secured with parafilm. The three-necked flask was charged with magnesium turnings (1.55g, 64 mmol), a small crystal of iodine and THF (25 mL). A small amount (~0.5 mL) of a THF solution (5 mL) of 2,3-dichloropropene (2.22 g, 20 mmol) was added to the magnesium, the mixture was stirred for about 10 min. and a very exothermic reaction ensued after slightly warming the reaction flask. The allene gas, generated, was bubbled into a solution of *n*-BuLi (0.65 mL, 1.70 mmol) in dry diethyl ether (5 mL) and dry hexanes (4.35 mL) [32], at -78° C, contained in the two-necked flask, under nitrogen atmosphere. The remaining THF solution of 2,3-dichloropropene was added in small portions, in order to maintain a vigorous generation of allene. It is important to keep a positive pressure of allene during all the process, otherwise a drop in pressure in the three-necked flask, would produce a vacuum in the second flask. To avoid loss of material, during the process of generation of allene, the valve connecting the two-necked flask (containing *n*-BuLi) to a manifold, was kept closed, and the allene was collected into a balloon. After generation of allene stopped, the double-tipped needle was removed from the flask, and the *n*-BuLi-allene solution was stirred at -78° C for 1 hour. After this time a white suspension was obtained, and it was assumed to be about 0.85 mmol of dianion 1,3-dilithiopropyne, **19**.

4.4. Synthesis of 1,3-enyne **11**:

4.4.1. Synthesis of *N*-(2,2-diethoxyethyl)-1-Naphthylmethylamine **16**:

To an oven-dried, 50 mL round-bottom flask, equipped with a magnetic stirring bar, under nitrogen atmosphere, was added 1-naphthylmethylamine (0.114 g, 0.7 mmol) and dry THF (3 mL). The solution was cooled to 0° C and a solution of *n*-BuLi in hexanes was added dropwise (0.30 mL, 0.75 mmol). After stirring for 30 minutes, a THF solution (3 mL) of bromoacetaldehyde diethyl acetal, **15**, (0.139 g, 0.70 mmol) was added and the resulting solution stirred for 18 hours at room temperature. After this time, a saturated NH₄Cl solution was added (5 mL) and it was extracted with ether (2x10mL). The organic phase was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography using a mixture ether:hexane 7:3, to give 0.179 g of product (94%). ¹H NMR (400 MHz, CDCl₃) δ: 1.19 (t, *J* = 7.09 Hz, 6H), 1.74 (s, 1H), 2.87 (d, *J* = 5.54 Hz, 2H), 3.60 (m, 4H), 4.27 (s, 2H), 4.65 (t, *J* = 5.53 Hz, 1H), 7.42 (dd, *J* = 8.07 Hz, 1H), 7.51 (m, 3H), 7.77 (d, *J* = 8.00 Hz, 1H), 7.86 (d, *J* = 7.95 Hz, 1H), 8.13 (d, *J* = 8.57 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ: 15.33, 51.63, 52.13, 62.46, 102.28, 123.78, 123.53, 125.74, 126.17, 126.18, 127.90, 128.83, 131.93, 134.04, 125.91; MS (EI) *m/z* (rel. intensity) 75 (23), 103 (39), 115 (21), 127 (4), 141 (100), 156 (14), 170 (7), 228 (5), 273 (2); HRMS (ESI, V⁺): *m/z* [M + H]⁺ calc. for C₁₇H₂₃NO₂ 274.1807, found 274.1810.

4.4.2. Synthesis of *N*-(2,2-diethoxyethyl)-*N*-methyl-1-Naphthylmethylamine **17**:

To an oven-dried 50 mL round-bottom flask, equipped with a magnetic stirring bar, under nitrogen atmosphere, was added *N*-(2,2-diethoxyethyl)-1-naphthylmethylamine (**16**) (0.30 g, 1.1 mmol) and dry THF (15 mL). The solution was cooled to 0°C and a solution of *n*-BuLi in hexanes was added dropwise (0.55 mL, 1.40 mmol). After stirring for 30 minutes, a THF (5 mL) solution of iodomethane, (0.195 g, 1.40 mmol) was added and the resulting solution stirred overnight. After this time, a saturated NH₄Cl solution was added (5 mL) and it was extracted with ether (2x10mL). The organic phase was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography using a mixture ether:hexane 4:6, to give 0.300 g of product (95%). ¹H NMR (400 MHz, CDCl₃): 1.18 (t, *J* = 7.11 Hz, 6H), 2.31 (s, 3H), 2.69 (d, *J* = 5.34 Hz, 2H), 3.55 (m, 4H), 3.98 (s, 2H), 4.69 (t, *J* = 5.34 Hz, 1H), 7.40 (m, 2H), 7.49 (m, 2H), 7.77 (d, *J* = 7.87 Hz, 1H), 7.84 (d, *J* = 8.10 Hz, 1H), 8.40 (d, *J* = 8.40 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): 15.43, 43.52, 60.32, 61.63, 61.46, 101.33, 125.14, 125.17, 125.78, 125.88, 127.64, 128.07, 128.41, 133.98, 135.06, 137.70; MS (EI) *m/z* (rel. intensity) 75 (8), 103 (8), 115 (21), 141 (100), 171 (10), 184 (51), 242 (6), 287 (2); HRMS (ESI, V⁺): *m/z* [M + H]⁺ calc. for C₁₈H₂₆NO₂ 288.1964, found 288.1961.

4.4.3. Synthesis of *N*-methyl-*N*-(1-naphthylmethyl)acetaldehyde **18**:

To an oven-dried, 50 mL round-bottom flask, equipped with a magnetic stirring bar, and a Liebig condenser, under nitrogen atmosphere, was added *N*-(2,2-diethoxyethyl)-*N*-methyl-1-Naphthylmethylamine (**17**) (0.062 g, 0.2 mmol), FeCl₃•6H₂O (0.190 g, 0.7 mmol), 3 mL of a mixture of CH₂Cl₂:acetone (4:1) and 0.3 mL HCl (6 mol/L), and the mixture was refluxed for 4 hours. The reaction mixture was allowed to cool to room temperature and a saturated solution of NaHCO₃ (5 mL) was added and extracted with ether (2 x 10mL). The organic phase was washed with water (3 x 10mL) and it was dried over Na₂SO₄ and concentrated *in vacuo*. The crude aldehyde was taken into the next step without any further purification.

4.4.4. (*E*)-*N*-Methyl-*N*-(1-naphthylmethyl)-2-penten-4-ynylamine **11**:

Crude acetaldehyde **18**, prepared as above, and assumed to be ~0.2 mmol, was dissolved in ether (3 mL) and added to a 1,3-dilithiopropyne solution, prepared as above (assumed to be 0.85 mmol) and under nitrogen. The mixture was stirred and allowed to reach room temperature in 3 hours. After this time tosyl chloride (0.275 g, 1.4 mmol) dissolved in dry THF (2 mL) and HMPA (0.80 mL, 4.6 mmol) was added and stirred at room temperature for 18 hours. The reaction mixture was

cooled to 0°C and treated with *tert*-BuLi in pentane (2.0 mL, 3.4 mmol) and stirred for three hours. The reaction was quenched by addition of ammonium chloride saturated solution (3-5 mL), extracted with ether (2 x 10 mL), dried over Na₂SO₄ and concentrated *in vacuo*. GC analysis of the crude reaction showed a 90% conversion. The crude reaction was purified by column chromatography (ether:hexane, 1:9) to give 0.010 g of product. ¹H NMR (400 MHz, CDCl₃): 2.24 (s, 3H), 2.85 (d, *J* = 2.41 Hz, 1H), 3.16 (d, *J* = 6.20 Hz, 2H), 3.91 (s, 2H), 5.68 (dd, *J* = 2.4, 15.8 Hz, 1H), 6.36 (dt, *J* = 6.3, 15.8 Hz, 1H), 7.40 (m, 2H), 7.50 (m, 2H), 7.78 (m, 1H), 7.85 (d, *J* = 8.1 Hz, 1H), 8.25 (d, *J* = 8.1 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 42.5, 59.3, 60.3, 82.1, 87.7, 111.2, 143.4, 124.7, 125.0, 125.2, 125.7, 126.0, 127.5, 128.2, 128.6, 129.7, 132.5; MS (EI) *m/z* (rel. intensity) 65 (9), 94 (14), 108 (7), 127 (5), 141 (100), 155 (22), 220 (18), 234 (20), 235 (5); HRMS (ESI, V⁺): *m/z* [M + H]⁺ calc. for C₁₇H₁₇N 236.1439, found 236.1441.

4.5. Synthesis of 4-(*trans*-1-Buten-3-ynyl) *N,N*-Dimethylaniline, **12**:

This enyne was prepared by reaction of 1,3-dilithiopropyne, **19**, with 4-dimethylaminobenzaldehyde, **22**, as described above. ¹H NMR (400 MHz, CDCl₃): 7.28 (d, *J* = 8.9 Hz, 2H), 6.97 (d, *J* = 16.3 Hz, 1H), 6.65 (d, *J* = 8.9 Hz, 2H), 5.93 (dd, *J* = 2.3, 16.3 Hz, 1H), 2.99 (s, 1H), 2.98 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): 151.02, 143.51, 127.72, 124.29, 112.20, 101.80, 84.21, 77.69, 40.43; MS (EI) *m/z* (rel. intensity) 77 (12), 115 (8), 127 (37), 128 (8), 141 (3), 155 (15), 171 (100); HRMS (ESI, V⁺): *m/z* [M + H]⁺ calc. for C₁₂H₁₄N 172.1126, found 172.1124.

4.6. Synthesis of enyne **13**

4.6.1. *tert*-Butyldimethylsilyl 2-Propynyl Ether, **25**:

In a 100 mL round bottom flask, was added homopropargyl alcohol, **24**, (0.56 g, 10 mmol), DMF (10 mL), *tert*-butyldimethylsilyl chloride (1.7 g, 13 mmol) and imidazole (0.17 g, 2.5 mmol), and the solution was stirred for 18 h at room temperature. After this time water (10 mL) was added to the mixture and extracted with ether (2 x 5 mL). The organic extracts were dried over magnesium sulphate and concentrated *in vacuo*. The residue obtained was purified by Kugelrohr distillation to obtain 1.65 g of product (97% yield). ¹H NMR (400 MHz, CDCl₃): 0.12 (s, 6H), 0.91 (s, 9H), 2.38 (t, *J* = 2.54 Hz, 1H), 4.30 (d, *J* = 2.54 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃): -5.06, 18.42, 25.93, 51.65, 72.98, 82.57; MS (EI) *m/z* (rel. intensity) 75 (18), 83 (100), 99 (5), 113 (100), 155 (4), 170 (2); HRMS (ESI, V⁺): *m/z* [M + H]⁺ calc. for C₉H₁₉OSi 171.1205, found 171.1205.

4.6.2. *tert*-Butyldimethylsilyl (*trans*-5-chloro-4-penten-2-ynyl) ether, **26**:

In a three necked, 50 mL, round bottom flask, under nitrogen atmosphere, were added PdCl₂(PPh₃)₂ (0.11 g,

0,15 mmol), *trans*-1,2-dichloroethene (2.90 g, 30 mmol) dry THF (20 mL) and, the resulting solution, was stirred at room temperature for 20 min. A diethylamine solution (4 mL) of acetylene, **25**, was added, followed by addition of CuI (0,06 g; 0,15 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction was quenched by addition of saturated ammonium chloride solution (5 mL), and extracted with ether (2 x 10 mL). The organic phase was dried over magnesium sulphate and concentrated *in vacuo*. The residue obtained was purified by column chromatography (ether:hexane, 1:1) to obtain 1.23 g of product (90% yield). ^1H NMR (400 MHz, CDCl_3): 0.12 (s, 6H), 0.91 (s, 9H), 4.41 (d, $J = 2.2$ Hz, 2H), 5.95 (dt, $J = 2.2$, 13.7 Hz, 1H), 6.51 (d, $J = 13.7$, 1H); ^{13}C NMR (100 MHz, CDCl_3): δ -5.00, 18.47, 25.96, 52.23, 79.98, 90.74, 113.64, 130.61; MS (EI) m/z (rel. intensity) 73 (12), 99 (8), 143 (100), 145 (33), 173 (58), 175 (18), 215 (2); HRMS (ESI, V^+): m/z $[\text{M} + \text{H}]^+$ calc. for $\text{C}_{11}\text{H}_{20}\text{OSiCl}$ 231.0972, found 231.0972.

4.6.3. Preparation of *p*-*N,N*-dimethylamino phenyl magnesium bromide, **27**

To a 50 mL three necked, round bottom flask, with a Liebig condenser, was added *N,N*-dimethylbromoaniline (0.82 g, 4 mmol), magnesium turnings (0.100 g, 4.3 mmol), a small iodine crystal and THF (4 mL). The mixture was refluxed for 30 min and a dark solution was obtained.

4.6.4. Preparation of *tert*-butyl dimethylsilyl-5-*trans*-(*p*-*N,N*-dimethylaminophenyl)-4-penten-2-ynyl ether, **28**:

In a dry round bottom flask was added $\text{Pd}(\text{PPh}_3)_4$ (0,150 g, 0,13 mmol), vinyl chloride **26** (0.573 g, 2.6 mmol) and dry THF (5 mL) and the mixture was stirred for 20 min. After this time Grignard, **27**, solution, previously prepared, was added dropwise, *via* canula, and the mixture stirred for 18 hours at room temperature. After this time, the reaction was quenched by addition of saturated ammonium chloride solution (5 mL) and extracted with ether (2 x 10 mL). The organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The residue obtained was purified by column chromatography (Et_2O :hexane, 1:9) to give 0.760 g of **28** (97 % yield). ^1H NMR (400 MHz, CDCl_3): 0.15 (s, 6H), 0.94 (s, 9H), 2.97 (s, 6H), 4.49 (d, $J = 2.1$ Hz, 2H), 5.95 (dt, $J = 2.1$, 16.2 Hz, 1H), 6.65 (d, $J = 8.9$, 2H), 6.86 (d, $J = 16.2$, 1H), 7.27 (d, $J = 8.9$, 2H); ^{13}C NMR (100 MHz, CDCl_3): -4.90, 18.51, 26.05, 40.45, 52.61, 85.15, 88.57, 102.82, 112.24, 124.69, 127.58, 141.78, 150.83; MS (EI) m/z (rel. intensity) 115 (24), 168 (15), 184 (100), 228 (25), 258 (9), 315 (49), 316 (13); HRMS (ESI, V^+): m/z $[\text{M} + \text{H}]^+$ calc. for $\text{C}_{19}\text{H}_{30}\text{NOSi}$ 316.2097, found 316.2091.

4.6.5. *trans*-5-(*p*-*N,N*-Dimethylaminophenyl)-4-penten-2-yn-1-ol, **29**:

To a THF solution (10 mL) of silyl ether, **28**, (0.370 g, 1.17 mmol) was added, at 0° C, a 1.0 M THF solution of

tetrabutylammonium fluoride (2.4 mL, 2.4 mmol) and the resulting solution stirred for 3.5 h. The reaction was quenched by addition of water (10 mL) and extracted with ether (2 x 10 mL). The organic phase was dried (MgSO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (Et_2O :hexane, 1:1) to give 0,201 g (85 % yield). ^1H NMR (400 MHz, CDCl_3): 3.00 (s, 6H), 4.46 (dd, $J = 2.2$, 6.1 Hz, 2H), 5.97 (dt, $J = 2.2$, 16.2 Hz, 1H), 6.65 (d, $J = 8.8$ Hz, 2H), 6.91 (d, $J = 16.2$ Hz, 1H), 7.27 (d, $J = 8.8$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ 40.44, 52.06, 86.17, 87.88, 102.23, 112.23, 124.47, 127.69, 142.44, 150.95; MS (EI) m/z (rel. intensity) 77 (5), 115 (11), 128 (26), 157 (17), 170 (16), 184 (15), 201 (100); HRMS (ESI, V^+): m/z $[\text{M} + \text{H}]^+$ calc. for $\text{C}_{13}\text{H}_{16}\text{NO}$ 202.1232, found 202.1230.

4.6.6. *trans*-5-(*p*-*N,N*-Dimethylaminophenyl)-4-penten-2-yn-1-ol, **13**:

To a 50 mL round-bottom flask was added Dess-Martin reagent (0.042 g, 0.1 mmol) and 5 mL CH_2Cl_2 . To this mixture was added alcohol **29** (0.010 g, 0.05 mmol) in CH_2Cl_2 (5 mL) and the resulting mixture stirred for 1 h. The reaction was quenched by addition of a $\text{Na}_2\text{S}_2\text{O}_3$ solution (5 mL) and NaHCO_3 and it was extracted with ether (2 x 10 mL). The organic extracts were dried over MgSO_4 , and concentrated *in vacuo*. The residue was purified by column chromatography (Et_2O :hexane, 9:1) to give 0.009 g of **13** (90% yield). ^1H NMR (400 MHz, CDCl_3): 3.03 (s, 6H), 6.03 (dd, $J = 1.2$, 16.1 Hz, 1H), 6.65 (d, $J = 8.9$ Hz, 2H), 7.25 (d, $J = 16.1$ Hz, 1H), 7.35 (d, $J = 8.9$ Hz, 2H), 9.31 (d, $J = 1.2$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3): 40.26, 91.09, 98.58, 111.95, 123.15, 129.10, 147.24, 150.32, 170.65, 176.58; MS (EI) m/z (rel. intensity) 77 (9), 115 (7), 127 (26), 128 (26), 140 (4), 155 (18), 170 (38), 198 (30), 199 (100); HRMS (ESI, V^+): m/z $[\text{M} + \text{H}]^+$ calc. for $\text{C}_{13}\text{H}_{13}\text{NO}$ 200.1075, found 200.1076.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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