European Journal of Medicinal Chemistry 155 (2018) 579–589

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

# Novel carbazole-triazole conjugates as DNA-targeting membrane active potentiators against clinical isolated fungi

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ARTICLE INFO

*Article history:*
Received 30 January 2018
Received in revised form
9 May 2018
Accepted 8 June 2018

*Keywords:*
Carbazole
Triazole
Antifungal
Resistance
DNA

ABSTRACT

A series of carbazole-triazole conjugates were designed, synthesized and characterized by IR, NMR, and HRMS spectra. Biological assay showed that most of the synthesized compounds exhibited moderate and even strong antifungal activities, especially 3,6-dibromocarbazolyl triazole **5d** displayed excellent inhibitory efficacy against most of the tested fungal strains (MIC = 2–32 μg/mL) and effectively fungicidal ability towards *C. albicans*, *C. tropicals* and *C. parapsilosis* ATCC 22019 (MFC = 4–8 μg/mL). Its combination use with fluconazole could enhance the antifungal efficacy, and compound **5d** also did not obviously trigger the development of resistance in *C. albicans* even after 10 passages. Preliminary mechanism study revealed that the active molecule **5d** could depolarize fungal membrane potential and intercalate into DNA to possibly block DNA replication, thus possibly exhibiting its powerful antifungal abilities. Conjugate **5d** could interact with HSA, which was constructive for the further design, modification and screening of drug molecules. Docking investigation demonstrated a non-covalent binding of **5d** with CYP51 through hydrogen bond and hydrophobicity. These results strongly suggested that compound **5d** could act as a potential template for the development of promising antifungal drugs.



## 1. Introduction

Fungal invasion has imposed an increasingly serious problem in clinical treatments. Pathogenic infections such as cryptococcosis, candidiasis and aspergillosis are especially dire due to the high morbidity and mortality [1]. There is a dearth of novel clinically approved antifungal drugs with specific activity, and currently marketed available drugs for the treatment of fungal infections are restricted to mainly five basic classes of agents: azoles, polyenes, fluoropyrimidines, allyamines and echinocandins, which mainly target ergosterol biosynthesis, impede ergosterol binding or inhibit the synthesis of the fungal-specific cell wall polymer β-1,3-glucan, respectively [2]. Along with the limited repertoire of the marketed antifungals, limitations such as pathogen resistance, narrow inhibitory spectra, chronic deleterious effects, low clinical efficacy thanks to poor pharmacokinetics, complicated drug interactions with host cells, and poor bioavailability also emerge as major challenges in search of novel structural synthetic antifungal agents [3], which underscores an alarming need to discover new antifungal agents with unique mechanism of action, potent fungicidal activity, target specificity, rapid time killing and the absence of cross-resistance. Drug resistance mechanisms mainly involve weak drug affinity, abundant targets, low intracellular drug concentrations caused by efflux pumps, and easy formation of biofilms [4]. It is also noteworthy that the discovery of antifungal drug is more challenging than the development of antibacterial drug since fungi have similar metabolism to mammalian cells except for cell wall, thus resulting in few pathogen specific targets [5].

Fluconazole has long been as front-line orally bioavailable drug in the prophylaxis and defense of both superficial and invasive fungal pathogens due to its favorable pharmacokinetics, excellent activity against *Candida* spp., and safety profile [6]. Mechanism investigations have suggested that fluconazole could target cytochrome P450 through the coordination of triazole ring with the iron (II) ion of heme to inhibit enzyme lanosterol 14 α-demethylase

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https://doi.org/10.1016/j.ejmech.2018.06.022
0223-5234/

(CYP51) in fungal ergosterol biosynthesis pathway and the tertiary alcohol moiety in fluconazole is competent to forming hydrogen bonds with the active site residue H310 [7]. However, the fungistatic fluconazole generally gives rise to severe mammalian toxicity and impedes it from treating deep-seated mycoses and life-threatening systemic infections [8]. The excessive reliance on fluconazole has also aggregated the appearance of resistant strains. Inspired by the clinical applicability, structural modification of fluconazole has lead to third-generation clinical azoles like voriconazole, posoconazole, isavuconazole [9], and this further promotes the exploition of non-clinical antifungal agents with amendatory therapeutic indexes [10].

Carbazole derivatives have attracted widespread attention as they have distinct features by mediating a wide range of biological activities. Natural carbazomycins and murrayafoline A have been successfully applied as antibiotics in market [11]. *N*-Alkylated carbazoles have been reported to exhibit diversified bioactivities [12] and particularly azole ring functionalized carbazoles at *N*-position are observed with comparable or even superior repressive activities towards the tested fungi to the reference drugs [13]. Additionally, the modification of the 3- and 6-positions of carbazole has also provoked the interests to exploit novel effective antifungal candidates [14] and the introduction of halogen to carbazole scaffold also plays important role in preventing lethal diseases from infectious pathogens [15]. It has been found that carbazoles with large π-conjugated backbone could not only target topoisomerase and non-covalently bind with DNA in an intercalative manner through base pairs, minor groove binding or electrostatic interactions [16], but also disrupt the integrity of the microbial membrane and misloc-alize essential membrane-associated protein [17]. Therefore, the exploration of potential carbazole-based antifungal agents with multiple targets has received widespread attention and provides an important step forward for this field with the expectation to overcome the drug resistance of current therapies.

So far little literature has reported the conjugation of carbazole to fluconazole backbone. Conjugates with two or more bioactive pharmacophores occupy a significant position in the discovery of new biological agents, which own new targets or different action mechanisms [18]. On the basis of our previous work on carbazole, we replaced one triazole ring in fluconazole by carbazole scaffold and remained triazolyl alcohol moiety to generate a series of carbazole-triazole conjugates as a new type of potential antifungal agents (Fig. 1), where the hydroxy group, nitrogen atom and aromatic triazole or carbazole ring might be competent to interact with active sites in biological system and simultaneously bind with several targets, thus hopefully overcoming drug resistance. The incorporation of halogen atom to pharmacophores has been acknowledged to be beneficial for bioactivities [19] and alkyl groups are prevalently accepted to be able to modify lipid-water partition coefficients by modulating lipophilicity, binding affinity or cell membrane permeability, which might influence the rate of *in vivo* absorption and transport of drugs [20]. Therefore, various halogens with varying degrees of electro-negativities and alkyl groups were introduced to carbazole scaffold and phenyl ring to investigate the influence on antifungal activities, which might decrease or eliminate the deleterious effects. Indole with a smaller conjugated system in comparison with carbazole is also considered to be a versatile fragment in medicinal chemistry, and shows immense potentiality in antimicrobial aspects [21]. Consequently, indole fragment was combined with fluconazole structure to study whether the shrinkage of conjugated skeleton made contribution to antifungal activities.

A series of carbazole-triazole conjugates as potential antifungal agents were designed, synthesized and characterized by IR, NMR, and HRMS spectra. Biological evaluation for *in vitro* antifungal, fungicidal, antibacterial activities and structure activity relationship (SAR) were discussed. Drug tolerance of fungi is usually accompanied with the overexpression of drug efflux pump genes, the change of membrane lipid fluidity, and the formation of biofilms [22]. Therefore, drug combination was implemented to overcome the tolerance. Molecular modeling was completed to explain the potential antifungal action, and fungal resistance of the highly active molecule was also evaluated. Possible antifungal mechanism exploration was performed by studying membrane depolarization potentiality and DNA intercalating ability. Further binding analysis between bioactive target molecules and human serum albumins (HSA) was instructive for the design, modification, and screening of drug molecules.

## 2. Results and discussion

### 2.1. Chemistry

The synthetic route of target carbazole type of fluconazole analogs was outlined in Scheme 1. The desired compounds were prepared *via* multistep reactions from commercially available substituted benzene, triazole, and various carbazoles. The intermediates **2a**–**e** could be efficiently prepared in satisfactory yields (80.1–92.3%) by the acetylation of substituted benzenes **1a**–**e** with chloroacetyl chloride, and further went through nucleophilic substitution by 1,2,4-triazole in acetonitrile with potassium carbonate as base to afford triazo1yl ethanones **3a**–**e** in good yields of 57.3–75.5%. The further epoxidation of compounds **3a**–**e** in dichloromethane by trimethyl sulfoxonium iodide (TMSOI) and 32%

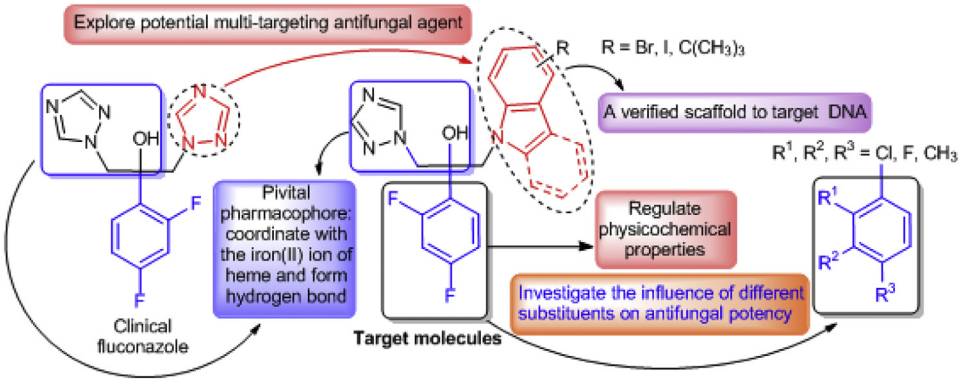


**Fig. 1.** Design of carbazole-triazole conjugates as potential antifungal agents.