Bioorganic & Medicinal Chemistry Letters 27 (2017) 4964–4969

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel potentially antifungal hybrids of 5-flucytosine and fluconazole: Design, synthesis and bioactive evaluation

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ARTICLE INFO

Article history:
Received 11 August 2017
Revised 6 October 2017
Accepted 8 October 2017
Available online 13 October 2017

Keywords:
5-Flucytosine
Fluconazole
Antifungal
Molecular modeling
Quantum chemical studies
DNA

ABSTRACT

A series of novel potentially antifungal hybrids of 5-flucytosine and fluconazole were designed, synthesized and characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS spectra. Bioactive assay manifested that some prepared compounds showed moderate to good antifungal activities in comparison with fluconazole and 5-flucytosine. Remarkably, the 3,4-dichlorobenzyl hybrid **7h** could inhibit the growth of *C. albicans* ATCC 90023 and clinical resistant strain *C. albicans* with MIC values of 0.008 and 0.02 mM, respectively. The active molecule **7h** could not only rapidly kill *C. albicans* but also efficiently permeate membrane of *C. albicans*. Molecular docking study revealed that compound **7h** could interact with the active site of CACYP51 through hydrogen bond. Quantum chemical studies were also performed to explain the high antifungal activity. Further preliminary mechanism research suggested that molecule **7h** could intercalate into calf thymus DNA to form a steady supramolecular complex, which might block DNA replication to exert the powerful bioactivities.

The development of drug-resistant fungal strains presents a serious threat to human health worldwide because conventional antifungal agents are becoming weak efficacy in treating fungal infections. Therefore, the increasingly active effort is to discover new antifungal agents. 5-Fluorocytosine is a synthetic antimycotic drug in basic health systems, however, 5-fluorocytosine has no intrinsic antifungal activity indeed, only after uptake by the fungal cells, this compound can be converted into 5-fluorouracil first, and further turned into metabolite 5-fluorodeoxyuridine, which inhibits the synthesis of RNA and DNA.[1] Unfortunately, 5-fluorocytosine as a single antifungal agent in clinical use is problematic owing to its relatively weak antifungal efficacy and fast development of resistance. Thus, the combinational use of 5-fluorocytosine with clinical antifungal drugs is a prevalent therapy for fungal infection. Amphotericin B with 5-fluorocytosine can treat severe systemic mycoses, such as *cryptococcosis*, *candidosis*, *chromoblastomycosis* and *aspergillosis*. Particularly, the combination of 5-fluorocytosine with azole-type antifungal agents such as fluconazole, itraconazole or ketoconazole exhibits excellent antifungal effects.[2] Attracted and inspired by such features, it is of great interest for us to employ 5-fluorocytosine as a constructing block to develop a series of hybrids of 5-fluorocytosine and fluconazole, and investigate their antifungal potencies.[3]

Fluconazole as one of the most important triazole antifungal drugs plays a significant role in prophylaxis and treatment of both superficial and invasive yeast fungal infections. It is a potent inhibitor of cytochrome P450 for the biosynthesis of ergosterol which is an essential component of the yeast cell membrane. Due to the good absorption and high oral bioavailability, fluconazole is the first choice of antifungal agent for treating infections caused by *Candida albicans* and *Cryptococcus neoformans* and has an exceptional therapeutic record including potent activity, excellent safety profile and favorable pharmacokinetic characteristics.[4] However, the excessive use of fluconazole has resulted in the increasing emergency of fluconazole-resistant *C. albicans* strains. Therefore, the development of resistant strains, the observed toxicity and the limited antifungal spectrum of fluconazole have created an urgent need to develop novel antifungal drugs with ameliorated potency, reduced adverse effects and completely novel targets. Currently, a large amount of effort has been directed towards structural modification of fluconazole to exploit fluconazole-based drugs with good efficiency, broad antimicrobial spectrum and amendatory therapeutic indexes.[5,6]

In the design of new drugs, the hybridization approach is a practical strategy worldwide to discover molecules with better activity

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https://doi.org/10.1016/j.bmcl.2017.10.020
0960-894X/

and less drug resistance due to the possibly different action mechanisms of hybrids.[7,8] Our previous work has reported some successful hybridization: a) clinafloxacin-fluconazole derivatives displayed broad antimicrobial spectrum, good antibacterial and antifungal activities with low MIC values ranging from 0.25 to 2 μg/mL; b) metronidazole-quinolones exhibited good or even stronger antimicrobial activities in comparison with reference drugs and were proven that the possible antibacterial mechanism might be in relation with multiple binding sites between bioactive molecules and topo IV–DNA complex.

Based on the above mentions and as an extension of our studies on the development of azole compounds,[9–11] we would like to combine the clinical antimycotic 5-fluorocytosine and antifungal fluconazole into one molecule for the first time to generate a series of novel hybrids **5**–**7** as potentially antifungal agents (Fig. 1). Various alkyl or aryl groups were introduced into the target hybrids to investigate the effects of different substituents on biological activities. Their antifungal activities were evaluated *in vitro* against five fungi strains. The fungicidal kinetic assay and membrane permeabilization of the highly active molecule were also evaluated. Moreover, the molecular modeling and experimental investigation of the highly active 5-fluorocytosine-fluconazole hybrid with DNA were further studied to explore the possible antifungal mechanism.

The synthetic route of target hybrids of 5-flucytosine and fluconazole was outlined in Scheme 1. The desired molecules were prepared *via* multistep reactions starting from commercially available materials 2,4-difluorobenzene, triazole and 5-flucytosine. The chloroacetylated intermediate **2** could be efficiently prepared in satisfactory yield of 65.8% by the acetylation of 2,4-difluorobenzene **1** with chloroacetyl chloride, and was then *N*-alkylated with 1,2,4-triazole in acetonitrile in the presence of potassium carbonate to afford the triazolyl ethanone **3** in good yield of 70.4%. Further epoxidation of compound **3** in dichloromethane by trimethyl sulfoxonium iodide (TMSOI), cetyltrimethylammonium bromide (CTAB) and 40% potassium hydroxide at 40–45 °C produced the triazolyl oxirane **4** in 68.2% yield[12]. The target molecule **5** was conveniently obtained in yield of 40.1% by the reaction of 5-flucytosine with compound **4** in ethanol using potassium carbonate as base at 80 °C. The *N*-alkylation of compound **5** with a series of saturated and unsaturated alkyl bromides in ethanol at 65 °C in the presence of potassium carbonate as base respectively afforded alkyl derivatives **6a**–**k** with yields ranging from 19.4 to 30.6%. The reaction of molecule **5** with halobenzyl halides produced the halophenyl derivatives **7a**–**h** in 20.3–30.8% yields. All the new compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS spectra.

All the newly prepared compounds were screened for their antifungal efficacies. Their minimum inhibitory concentration (MIC) *in vitro* was determined according to the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) using the two folds serial dilution method in 96-well microtest plates.[13] The tested fungi included two standard fungal strains (*Candida albicans* ATCC 90023, *Candida parapsilosis* ATCC 22019) and three clinical fungal strains (*Candida albicans*, *Aspergillus fumigatus*, *Candida tropicals*) with the positive controls of clinical antifungal fluconazole and 5-flucytosine. The antifungal data were summarized in Table 1.

The antifungal evaluation *in vitro* displayed that some prepared hybrids showed good bioactivities against the tested fungal strains (Table 1). Remarkably, 5-flucytosine-fluconazole hybrid **5** exhibited moderate to good antifungal activities with MIC values ranging from 0.04 to 0.17 mM in comparison with clinical drugs, which suggested that the hybridization of 5-flucytosine and fluconazole was favorable for the antifungal potentiality. According to this evidence, various substituents including alkyl and aryl groups were further introduced to modify 5-flucytosine-fluconazole hybrid **5** in order to illuminate the structure-activity relationships.

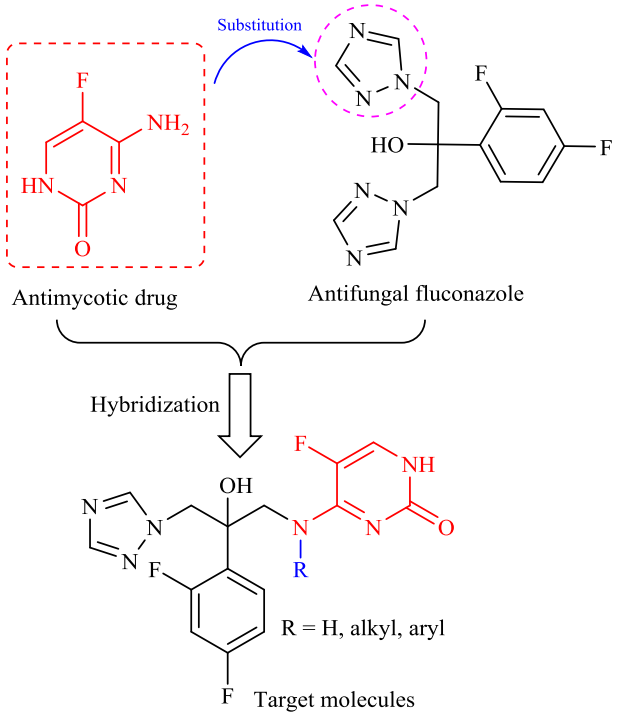

Fig. 1. Design of novel target hybrids of 5-flucytosine and fluconazole.

The replacement of hydrogen atom in the NH bridge of compound **5** with ethyl group led to compound **6a** with lower antifungal efficacy. Further extension of the carbon chain gave a series of derivatives **6b**–**i** with weak or no obviously antifungal activities in comparison with compound **5** and fluconazole, while the antifungal activity of ethyl analogue **6b** was superior to that of 5-flucytosine towards all the tested fungal pathogen. Specially, alkyl compounds **6a**–**i** showed good anti-*C. tropicals* and anti-*A. fumigatus* activities (except for hybrids **6f** and **6g**) in contrast with 5-flucytosine. These results revealed that the length of alkyl chain seemed to have weak effect on biological activities. Propyl analogue **6b** had moderate bioactivities against the tested fungal strains with MIC values ranging from 0.31 to 0.62 mM. Meanwhile, propyl chain was replaced by the unsaturated alkyl groups to produce compounds **6j** and **6k**, which exerted approximately the same antifungal activity in comparison with propyl hybrid **6b**, while their antifungal activities against three clinical fungal strains were better than those of 5-fluorocytosine and fluconazole (except for fluconazole against *C. albicans*). These data suggested that the introduction of saturated or unsaturated alkyl substituents exhibited weak effect on the antifungal potencies.

In comparison with alkyl derivatives **6a**–**k**, most of halobenzyl compounds **7a**–**h** gave relatively stronger activities in inhibiting the growth of the tested strains. Noticeably, in this halobenzyl series, compound **7h** with 3,4-dichlorobenzyl group exerted the best activities with MIC values of 0.008–0.03 mM, especially, it could inhibit the growth of *C. albicans* ATCC 90023 and clinical resistant strain *C. albicans* with MIC values of 0.008 and 0.02 mM, respectively. Moreover, its anti-*C. tropicals* (MIC = 0.03 mM) and anti-*A. fumigatus* (MIC = 0.03 mM) activities were superior to those of fluconazole and 5-fluorocytosine. For clinical *C. albicans* strains, it also showed eight times more active than fluconazole and 5-fluorocytosine. The substitution of 3,4-dichlorobenzyl moiety by 4-chlorobenzyl group yielded analogue **7d**, which showed stronger bioactivities against three clinical fungal strains with MIC values