European Journal of Medicinal Chemistry 64 (2013) 329–344

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Design, synthesis and antimicrobial evaluation of novel benzimidazole type of Fluconazole analogues and their synergistic effects with Chloromycin, Norfloxacin and Fluconazole

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ARTICLE INFO

Article history:
Received 25 January 2013
Received in revised form
20 March 2013
Accepted 24 March 2013
Available online 9 April 2013

Keywords:
Benzimidazole
Antibacterial
Antifungal
Fluconazole
Norfloxacin
Chloromycin

ABSTRACT

A novel series of benzimidazole type of Fluconazole analogues were synthesized and characterized by ^{1}H NMR, ^{13}C NMR, IR, MS and HRMS spectra. All the new compounds were screened for their antimicrobial activities *in vitro* by two-fold serial dilution technique. The bioactive evaluation showed that 3,5-bis(trifluoromethyl)phenyl benzimidazoles gave comparable or even stronger antibacterial and antifungal efficiency in comparison with reference drugs Chloromycin, Norfloxacin and Fluconazole. The combination of 2,4-difluorobenzyl benzimidazole derivative **5m** and its hydrochloride **7** respectively with antibacterial Chloromycin, Norfloxacin or antifungal Fluconazole showed better antimicrobial efficiency with less dosage and broader antimicrobial spectrum than the separated use of them alone. Notably, these combined systems were more sensitive to Fluconazole-insensitive *Aspergillus flavus* and methicillin-resistant MRSA.

1. Introduction

Fluconazole is a well known first-line antifungal drug recommended by World Health Organization and is prevalently used to treat fungal infections by *Candida albicans* and *Cryptococcus neoformans* with an exceptional therapeutic record including good activity and favourable pharmacokinetic characteristics. It is commonly considered that the triazole ring in Fluconazole could efficiently coordinate with the iron(II) ion of heme to restrain the biosynthesis of ergosterol and thus inhibiting the growth of fungi. However, this action generally leads to severe toxicity and precludes its application in the treatment of deep-seated mycoses and life-threatening systemic infections [1]. Moreover, its increasingly serious drug resistance, narrow antifungal spectrum and low activity against non-*Candida* fungi have attracted a large amount of effort towards further researches of Fluconazole in order to develop new more effective antifungal agents with broader antimicrobial spectrum and better therapeutic indexes [2–5]. Currently, the related work is mainly involved in the development of its prodrugs, structural modification of the side chains and exploitation of its analogues with new structural skeletons. Especially, the exploration of new leading compounds with wholly new structural molecules similar to the basic backbone of Fluconazole has become one of the predominant directions [4,6,7]. Some Fluconazole analogues such as Ravuconazole, Albaconazole and Isavuconazole have been successfully developed as clinical drugs for the treatment of fungal infections [8,9].

It is well known that tertiary amine moiety is an important structural fragment in bioactive compounds. This type of molecular fragment is able to form hydrogen bonds, coordinate with metal ions and accept protons and perform quaternization, and could not only regulate physicochemical properties of desired molecules but also interact with various enzymes and receptors in biological system which exerts broad bioactive spectrum. Therefore, tertiary amine fragment is widely present in clinical drugs like analgesic Methadone Hydrochloride and Fentanyl Citrate, antiallergic Cetirizine, antitumour Melphalan and Cyclophosphamide and so on [10]. Recently, a tertiary amine type of Fluconazole analogues has been found to show large potential as new type of antimicrobial

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http://dx.doi.org/10.1016/j.ejmech.2013.03.049

agents in which the tertiary alcohol moiety in Fluconazole was replaced by a tertiary amine fragment that may exert the same function with the active site residue H310 as the tertiary alcohol moiety in Fluconazole [11,12]. Like Fluconazole, this tertiary amine type of bis-triazole and bis-imidazole derivatives exhibited good antifungal activity. Interestingly, they also gave promising antibacterial efficiency [13,14]. This compels us with overwhelming interest to continuously investigate this tertiary amine type of special Fluconazole analogues. Benzimidazole is a fused ring of imidazole with benzene having larger conjugated system and electron richer properties than either triazole or imidazole, this unique structure endows its derivatives to possess extensive potential in medicinal chemistry especially antimicrobial aspect [15]. Herein we would like to develop this tertiary amine type of benzimidazole derivatives (Fig. 1) as wholly new structure type of antimicrobial agents which were expected to play large roles in the treatment of disease-caused fungi and bacteria.

The special benzene-fused imidazole ring could readily interact with various active targets in biological system *via* diverse noncovalent interactions like hydrogen bonds, coordination, ion-dipole, cation–π, π–π stacking and hydrophobic effect as well as van der Waals force, therefore benzimidazole-based derivatives exhibit various bioactivities [16–19]. So far many benzimidazole compounds have been successfully developed as clinical drugs such as antihistaminic Astemizole, anti-anabrotic Omeprazole, antiparasitic Albendazole and antihypertensive Candesartan, and have been prevalently used in treatment of various diseases. A large number of researches have been directing towards benzimidazole-based medicinal drugs and recently a lot of work has shown that benzimidazoles possess much potential to inhibit the growth of bacterial and fungal strains [20]. Benzimidazole is structurally similar to purine, and its derivatives could compete with purines, resulting in the distinct inhibition of the synthesis of nucleic acids and proteins inside the bacterial cell wall, thereby killing the bacterial strains or inhibiting their growth. These observations showed that the mechanism of benzimidazole type of antibacterial agents was different from that of well known classes of antibacterial drugs, thus development of benzimidazole derivatives as novel antibacterial agents may be served as a good strategy to overcome bacterial resistances. Notably, benzimidazole ring has been actively employed to modify natural antibiotics like macrocyclic lactones and β-lactams and synthetic antibacterial drugs [15]. The benzimidazole-modified derivatives usually exhibited much better antibacterial efficacy than their corresponding precursors, especially benzimidazole-modified antibacterial fluoroquinolone (**BMAF**) with piperazine type of tertiary amine fragment also showed significant activity against *C. albicans* [21]. More importantly, some structurally simple benzimidazole compounds like benzimidazole amines **BA** and **ZSL** gave strong antimicrobial efficacy [19,22,23]. Especially high anti-*Klebsiella pneumoniae* activity was observed for compound **BA** with MIC value of 0.5 μg/mL, nearly comparable to the reference drug Cefoperazone, and compound **ZSL** gave strong inhibitory activity against MRSA (MIC = 2 μg/mL) (Fig. 2). All these clearly point out that benzimidazole derivatives

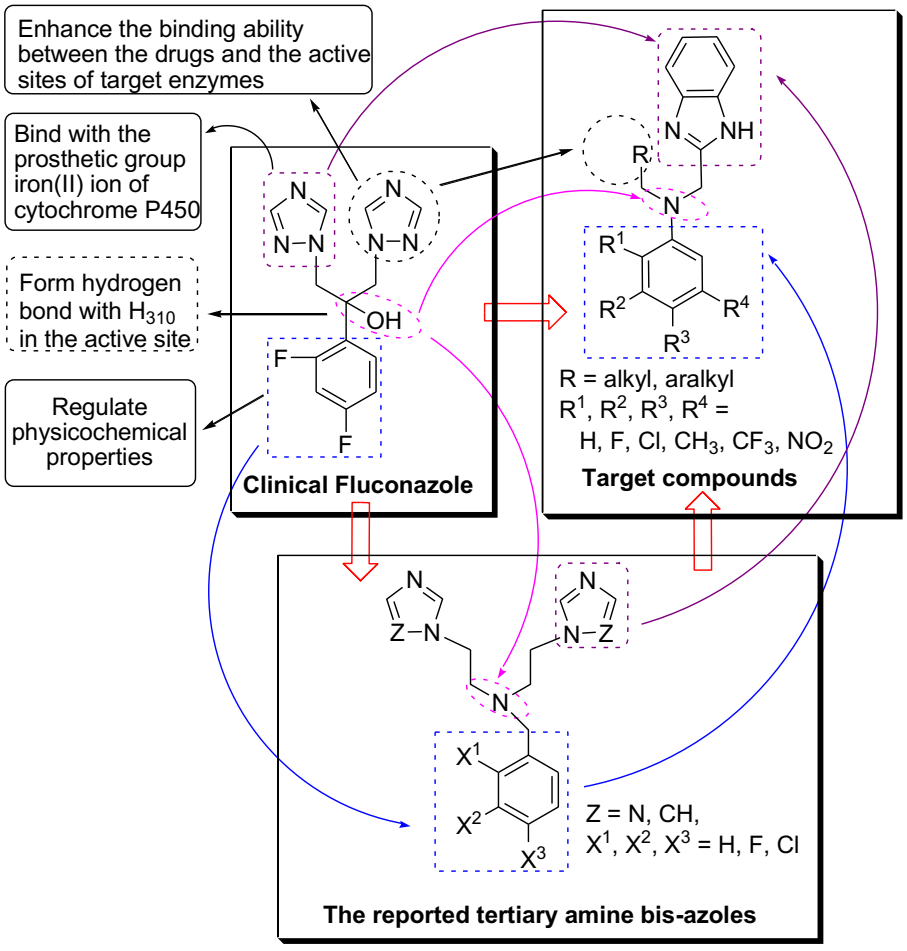

Fig. 1. Design of benzimidazole tertiary amine type of Fluconazole analogues.