Bioorganic & Medicinal Chemistry Letters 28 (2018) 2250–2255

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Benzofuro[3,2-*d*]pyrimidines inspired from cercosporamide *Ca*Pkc1 inhibitor: Synthesis and evaluation of fluconazole susceptibility restoration

Viet Hung Dao[a], Isabelle Ourliac-Garnier[b], Marc-Antoine Bazin[a], Catherine Jacquot[b], Blandine Baratte[c], Sandrine Ruchaud[c], Stéphane Bach[c], Olivier Grovel[d], Patrice Le Pape[b], Pascal Marchand[a,*]

[a] *Université de Nantes, Nantes Atlantique Universités, Département de Chimie Thérapeutique, EA1155 – IICiMed, Institut de Recherche en Santé 2, F-44200 Nantes, France*
[b] *Université de Nantes, Nantes Atlantique Universités, Département de Parasitologie et Mycologie Médicale, EA1155 – IICiMed, Institut de Recherche en Santé 2, F-44200 Nantes, France*
[c] *Sorbonne Université, CNRS, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), USR Protein Phosphorylation and Human Diseases, Station Biologique, Place Georges Teissier, F-29688 Roscoff, France*
[d] *Université de Nantes, Nantes Atlantique Universités, EA2160 – Mer Molécules Santé, Faculté de Pharmacie, F 44035 Nantes, France*

ARTICLE INFO

Keywords:
Cercosporamide
Benzofuro[3,2-*d*]pyrimidine
Antifungal activity
Candida albicans Pkc1
Fluconazole susceptibility restoration

ABSTRACT

In a context of growing resistance to classical antifungal therapy, the design of new drugs targeting alternative pathways is highly expected. Benzofuro[3,2-*d*]pyrimidine derivatives, derived from (−)-cercosporamide, were synthesized and evaluated as potential *Candida albicans* PKC inhibitors in the aim of restoring susceptibility to azole treatment. Co-administration assay of benzofuropyrimidinedione **23** and fluconazole highlighted a synergistic effect on inhibition of cell growth of a *Candida albicans* resistant strain.

Invasive fungal infections are a major cause of mortality worldwide, especially among vulnerable patients.[1] Among the 35,876 invasive fungal disease cases identified in France on the 2001–2010 period, 43.4% are candidemia; this number increases among patients with hematologic malignancies and those with chronic renal failure.[2] *Candida albicans* remains the most prevalent species in invasive candidiasis in the United States[3] and Europe.[4] It is noteworthy that the proportion of infections caused by non-*albicans Candida* (NAC) species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* has increased over the last two decades, species involvement depending on the infection site and the geography.[5] *Candida* pathogenicity is caused by a large number of mechanisms,[6] such as adherence to the host tissues and medical devices (biofilm), host recognition through binding to host cells and proteins and production of extracellular hydrolytic enzymes.[7] Its virulence depends on fungal but also on host factors in opportunistic situations.

In models of experimental infection, strains deleted for elements of MAPK-mediated signal transduction pathways exhibit a reduction or loss of virulence[8] and decrease in biofilm formation.[9] In *Candida albicans*, protein kinase C (called *Ca*Pkc1), one of the key proteins involved in MAPK pathways, is described as a regulator of cell wall integrity during growth, morphogenesis and response to cell wall stress.[10] In addition, La Fayette et al.[11] established a new role for PKC signaling in drug tolerance mechanisms. Given the limited number of antifungals used in clinic staments[12] and the emergence of drug resistance,[13] there is an urgent need to identify alternative targets in order to speed up the development of new generation of antifungals either more effective or able to restore susceptibility to classical antifungal drugs.[14] In this context, targeting PKC-mediated signal transduction pathway represents an new attractive strategy for antifungal therapy.[15]

(−)-Cercosporamide is a natural product isolated from the phytopathogen fungus *Cercosporidium henningsii*.[16] It was identified as a broad-spectrum antifungal agent displaying *in vitro* mean MIC value of 89 μg/mL[17] and of 10 μg/mL[18] against *C. albicans*. Interestingly, it appeared to act as a potent *Ca*Pkc1 ATP-competitive inhibitor with an IC_{50} of 44 nM.[18] Furthermore, cercosporamide inhibited human PKCα (IC_{50} = 1 μM) and PKCβ (IC_{50} = 0.3 μM)[18] and was later shown to inhibit other human kinases, including Mnk1/2, Jak3, GSK3β, ALK4 and Pim1, from nanomolar to low micromolar ranges.[19,20]

To the best of our knowledge, heterocyclic compounds displaying antifungal activity associated with *Ca*Pkc1 inhibition are not highlighted in the literature, except cercosporamide.[18] Consequently, in continuation of our successful attempts in the search of biologically active cercosporamide inspired derivatives,[21] we report here the

* Corresponding author.
E-mail address: pascal.marchand@univ-nantes.fr (P. Marchand).

https://doi.org/10.1016/j.bmcl.2018.05.044
Received 28 March 2018; Received in revised form 20 May 2018; Accepted 23 May 2018
Available online 23 May 2018
0960-894X/

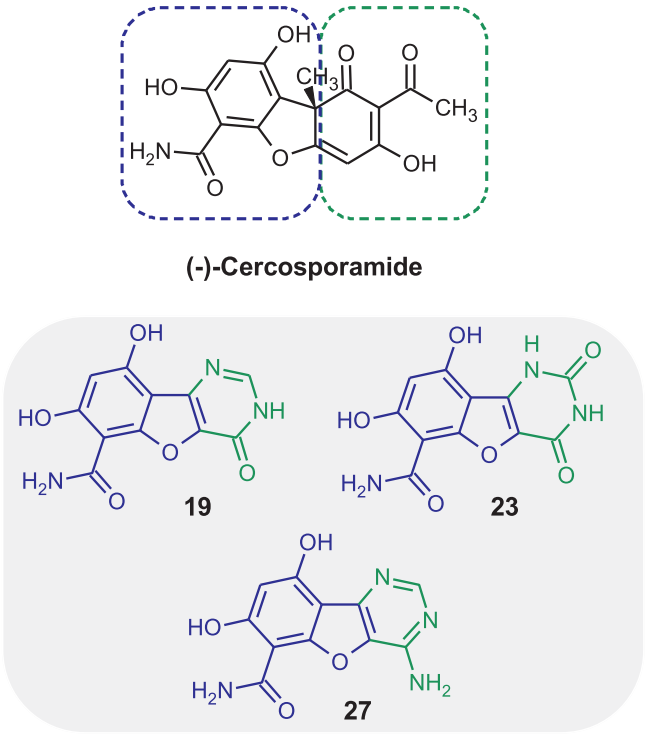


Fig. 1. Design of target compounds.

synthesis and antifungal evaluation of benzofuro[3,2-*d*]pyrimidines targeting *Ca*Pkc1.

The strategy of mimicking natural product for the design of antifungal agents to combat fungal resistance was very recently validated by the discovery of xanthones derived from α-mangostin.[22] In addition, cercosporamide was found to recognize ATP-binding site of Mnk2 kinase through hydrogen bond network due to the 3-OH and the 4-$CONH_2$ of the phenyl portion, justifying the strategy of keeping dihydroxybenzofuran-carboxamide part of the natural product model for the design of new tricyclic compounds (Fig. 1).[20]

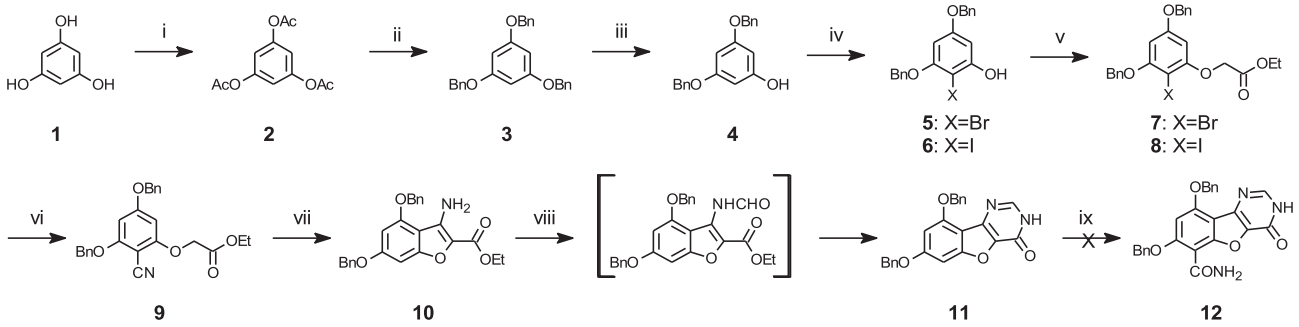
In addition, our expertise in the synthesis of pyrimidine-fused heterocycles as biological agents targeting kinases was used for the design of the new compounds, constituting the third ring of the benzofuro[3,2-*d*]pyrimidine derivatives described in this study.[23]

Benzofuran scaffold was first built from phloroglucinol **1** to achieve the suitable substitutions on the benzene ring and at the positions 2 and 3 for subsequent ring closure sequence (Scheme 1). 3,5-Dibenzyloxyphenol **4** was obtained in three steps by an initial tribenzylation of triacetoxyphloroglucinol **2** to avoid additive *C*-benzylation by direct *O*-benzylation of phloroglucinol **1**.[21,24] Mono-deprotection was carried out under transfer hydrogenation conditions using Pd/C and cyclohexene, in a mixture of ethyl acetate/ethanol at reflux, to afford compound **4** in a moderate yield (Scheme 1).[25] 2-Halo-3,5-dibenzyloxyphenols **5** and **6** were then synthesized by a mono-bromination or a mono-iodination procedure, respectively, very quickly at low temperature.[26] Afterwards, *O*-alkylation was realized using NaH as a base and ethyl bromoacetate at room temperature providing esters **7** and **8** in excellent yields.[27] In the next step, cyanation in the presence of copper cyanide in DMF gave ester **9** in a good yield from iodinated precursor **8** but the corresponding reaction, from the brominated counterpart **7**, remained less effective (19% of yield).[23b] Cyclization of the ethoxycarbonylmethylether **9** was performed in the presence of NaH to furnish benzofuran derivative **10** bearing amino group at position 3 and ester function at position 2.[28] Finally, benzofuro[3,2-*d*]pyrimidin-4-one derivative **11** was obtained through a ring closure reaction from benzofuran precursor **10** *via* a formamide intermediate reacting with ammonia.[27] Unfortunately, the carbamoyl group in position 6 could not be incorporated directly by electrophilic aromatic substitution using chlorosulfonyl isocyanate (CSI) followed by an acidic hydrolysis step.[21]

To circumvent this issue, we decided to introduce $CONH_2$ group at the beginning of the synthesis (Scheme 2). A direct aminocarbonylation of phenol derivative **4** in the presence of CSI afforded benzamide **13** after hydrolysis of the corresponding chlorosulfonyl intermediate. Afterwards, the same reaction sequence, as previously described in scheme 1, was applied to obtain benzofuro[3,2-*d*]pyrimidin-4-one derivative **12** bearing the carbamoyl appendage at C-6 position of the azaheterocycle. The only difference was the possibility to perform the mono-iodination at 0 °C *vs* -78 °C and according to the yields, it was better to carry out first the cyanation (compound **17**) and then *O*-alkylation (compound **16**). In the last step, benzyl cleavage was accomplished with concentrated sulfuric acid to provide the target compound **19**.

The synthetic route developed for the preparation of the 2,4-dioxo-1,2,3,4-tetrahydro[1]benzofuro[3,2-*d*]pyrimidine-6 carboxamide **23** is outlined in Scheme 3. To this end, *O*-alkylation of the benzamide derivative **17** was performed by the formation of the sodium salt in the presence of sodium hydride as base followed by its reaction with 2-iodoacetamide as described for the corresponding ester **16** (Scheme 2) but warming the medium at 60 °C.[29] Cyclization using KOH/EtOH at 60 °C furnished benzofuran-2,7-dicarboxamide **21** in acceptable yield. Indeed, we observed that these basic conditions were more efficient than NaH/DMF combination (50% *vs* 60% of yield).

Benzofuropyrimidinedione derivative **22** was then synthesized from benzofuran precursor **21** *via* an urea intermediate.[30] Finally, the dihydroxy analogue **23** was formed by treatment with concentrated sulfuric acid as procedure of debenzylation.



Scheme 1. (i) Ac_2O, pyridine, 120 °C, 5 h, 87%; (ii) BnCl, NaH, DMF, H_2O, 0 °C to rt, 10 h, 96%; (iii) C_6H_{10}, Pd-C 10%, AcOEt/EtOH (3/1), 110 °C, 2 h, 45%; (iv) NBS (1.0 eq.), CH_2Cl_2, −78 °C, 2 min, 95% for **5** or NIS (1.0 eq.), CH_2Cl_2, −78 °C, 2 min, 76% for **6**; (v) NaH, $BrCH_2CO_2Et$, DMF, rt, 12 h, 83% for **7** and 96% for **8**; (vi) CuCN, DMF, 160 °C, 1 h, 19% from **7** and 92% from **8**; (vii) NaH, DMF, 0 °C, 30 min, 66%; (viii) $HC(OEt)_3$, MW, 200 °C, 15 min then NH_3/MeOH 7 N, MW, 140 °C, 15 min, 31%; (ix) CSI, CH_3CN, rt, 24 h then HCl 1 N, rt, 24 h, failure.