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Piperazinyl quinolines as chemosensitizers to increase fluconazole susceptibility of *Candida albicans* clinical isolates

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ABSTRACT

The effectiveness of the potent antifungal drug fluconazole is being compromised by the rise of drug-resistant fungal pathogens. While inhibition of Hsp90 or calcineurin can reverse drug resistance in *Candida*, such inhibitors also impair the homologous human host protein and fungal-selective chemosensitizers remain rare. The MLPCN library was screened to identify compounds that selectively reverse fluconazole resistance in a *Candida albicans* clinical isolate, while having no antifungal activity when administered as a single agent. A piperazinyl quinoline was identified as a new small-molecule probe (ML189) satisfying these criteria.

Acquired drug resistance by medically relevant microorganisms poses a grave threat to human health and has enormous economic consequences.[1–3] Fungal pathogens present a particular challenge because they are eukaryotes and share many of the same mechanisms that support the growth and survival of the human host cells they infect. While contemporary antifungal medications such as fluconazole remain effective, the usefulness of such drugs is compromised by either dose-limiting host toxicity or the frequent emergence of high-grade resistance.[2,3]

The opportunistic fungus *Candida albicans* preferentially invades immunocompromised individuals and is responsible for numerous cutaneous, mucosal, and systemic blood-borne infections annually.[4] The azole antifungal fluconazole is often prescribed to control such infections, but fluconazole's fungistatic nature and emerging resistance are beginning to detract from its effectiveness. Typically, a daily regimen of 100 mg is sufficient to treat infections, but dosages as high as 800 mg/day can be ineffective against fluconazole-resistant *C. albicans*.[5]

Sensitizing *C. albicans* to fluconazole with small molecules is one approach to combat the emerging resistance of this pathogen. A limited number of small molecules have demonstrated modest potential in this arena,[6–11] and the proteins Hsp90 and calcineurin appear integral to some of the resistance pathways utilized by *Candida*.[12] This tentative progress towards stemming the increasing fluconazole resistance of *Candida* prompted us to screen the National Institutes of Health Molecular Libraries Probe Production Centers Network (NIH-MLPCN) compound collection with the goal of identifying small molecules that act as fungal-selective chemosensitizers and could be used to probe the various antifungal resistance mechanisms of *Candida*.

A high throughput screen of ~300,000 compounds evaluated growth inhibition of the *C. albicans* clinical isolate CaCi-2[5] in the presence of a sub-lethal concentration of fluconazole (Fig. 1, PubChem AID 1979).[13,14] 1,893 compounds exhibited >75% inhibition when dosed at 9.5 μM, of which 622 possessed IC_{50} values less than 1 μM when tested in a dose-response assay.

An orthogonal screen evaluated the efficacy of these 622 hits in combination with fluconazole against a more resistant *C. albicans* clinical isolate CaCi-8,[5,13] selecting for compounds that were active

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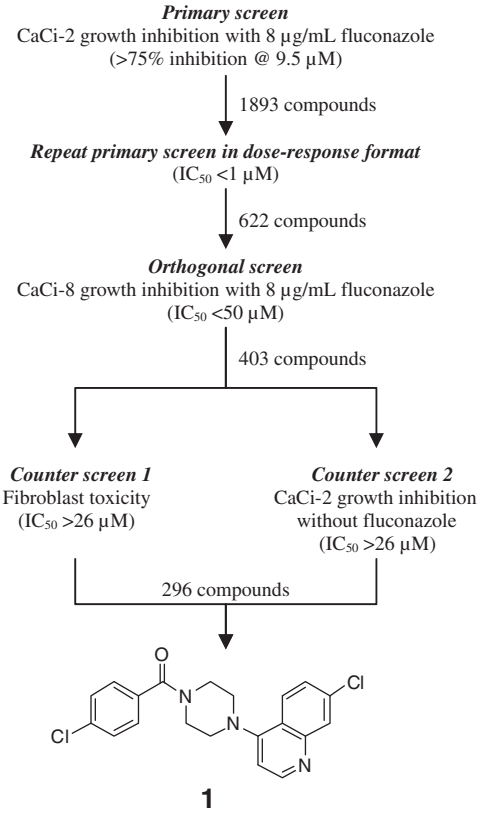


Figure 1. Summary of HTS campaign of the MLPCN ~300,000 compound collection. The selection criterion for each assay is given in parentheses.

with IC_{50}'s below 50 μM. At this stage, 403 compounds were identified as chemosensitizers of both CaCi-2 and CaCi-8.

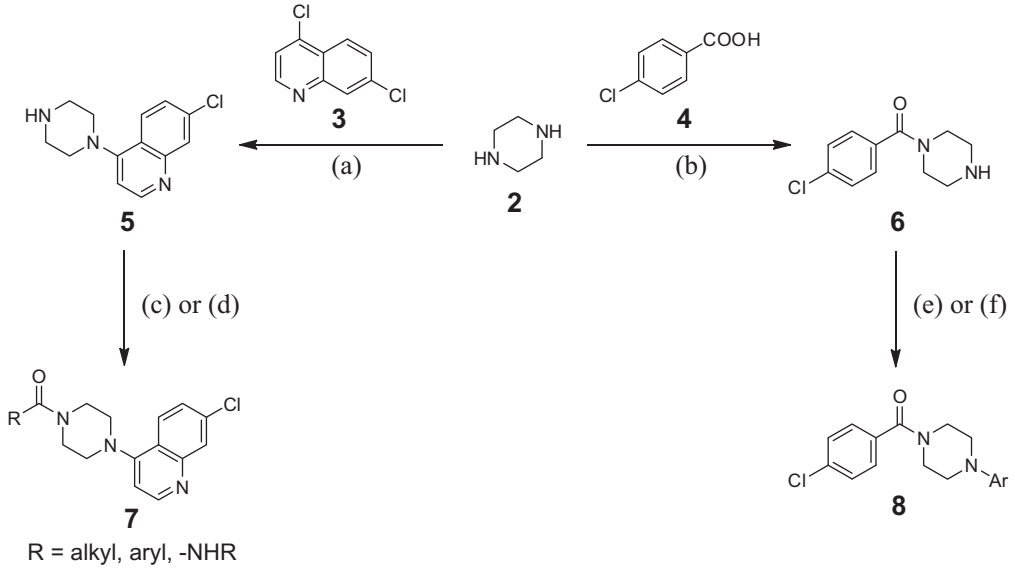
To remove hits with undesirable activity profiles, two counter screens were incorporated into the late stages of the screening campaign. The first employed murine 3T3 fibroblasts to assay non-selective mammalian cell toxicity, while re-evaluating CaCi-2 in the absence of fluconazole identified inherently fungitoxic substances. 296 of 403 candidates successfully passed both counter screens, and after examining these hits for chemical tractability, the piperazinyl quinoline **1** (Fig. 1) was selected for further investigation as a potential probe.

A number of analogs structurally related to **1** were prepared and evaluated for their ability to reverse fluconazole resistance in the *C. albicans* test strains. Two critical intermediates **5** and **6** were prepared by reacting excess piperazine with either 4-chlorobenzoic acid or 4,7-dichloroquinoline (Scheme 1). Amide coupling of piperazinyl quinoline **5** with different carboxylic acids afforded analogs **7**. Similarly, acylated piperazine **6** was appended to various aryl chlorides and bromides to provide analogs **8**.

Upon their preparation, the resulting collection of analogs was tested for their ability to increase fluconazole susceptibility in CaCi-2 and CaCi-8. The fungi were incubated at 37 °C for 48 h with the test compound and 8 μg/mL (26 μM) fluconazole before growth inhibition was assessed by Alamar blue fluorometry. Geldanamycin, a non-selective Hsp90 inhibitor, was used as a control for growth inhibition (100% inhibition at 10 μM).[12] The different analogs were also screened against mammalian fibroblasts and CaCi-2 in the absence of fluconazole to identify substances with intrinsic toxicity or antifungal effects. In the absence of fluconazole, none of the analogs showed any appreciable activity against CaCi-2 (IC_{50} = 15–26 μM) and were non-toxic to fibroblasts as well (IC_{50} = 21–26 μM).

The chemosensitizing properties of select 4-chlorobenzamide analogs **7** on CaCi-2 and CaCi-8 are presented in Table 1. The initial hit **1** proved to be an effective chemosensitizer of both fungal strains (IC_{50} = 0.7 and 1.3 μM, respectively) but suffered from poor solubility. Attempts to incorporate alternative *para*-substituents typically lowered the effectiveness of the resulting compounds as demonstrated with **7a–c**. Similarly, relocation of the chloro substituent to the *meta*- or *ortho*-positions (e.g., **7d–e**) was not tolerated, although the 3,4-dichloro variant **7f** displayed levels of activity comparable to the original hit.

Amides derived from alkanoic acids were significantly more soluble than **1** but were less effective chemosensitizers. Analogs bearing linear (**7h**), branched (**7i**), and cyclic (**7j–k**) alkyl chains were partial growth inhibitors of CaCi-2 at micromolar concentrations. However, the cycloalkane amides **7j–k** displayed greater effectiveness against the more resistant CaCi-8 strain than their acyclic counterparts (**7h–i**). Ureas such as **7l** were prepared from reacting



Scheme 1. Synthesis of analogs. Reagents and conditions: (a) Et_3N, 130 °C; (b) EDCI, DMAP, CH_2Cl_2; (c) R-CO_2H, EDCI, DMAP, CH_2Cl_2; (d) RNCO, CH_2Cl_2; (e) ArCl, Et_3N, 130 °C; (f) ArBr, NaO*t*-Bu, 15 mol % BINAP, 5 mol % $Pd_2(dba)_3$, toluene, 80 °C.