Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5127–5131
Bioorganic & Medicinal Chemistry Letters

# Quinazolinone-based fungal efflux pump inhibitors. Part 1: Discovery of an (*N*-methylpiperazine)-containing derivative with activity in clinically relevant *Candida* spp.

Rémy C. Lemoine,[a,*] Tomasz W. Glinka,[a] William J. Watkins,[a,†] Aesop Cho,[a] Jessie Yang,[a] Nadeem Iqbal,[b] Rajeshwar Singh,[b] Deidre Madsen,[a] Karen Lolans,[a] Olga Lomovskaya,[a] Uma Oza[a] and Michael N. Dudley[a]

[a]*Essential Therapeutics, Inc., 850 Maude Ave., Mountain View, CA 94043, USA*
[b]*NAEJA Pharmaceutical Inc., #2 4290-91 A St., Edmonton, Alberta, Canada T6E 5V2*

Received 18 May 2004; accepted 28 July 2004
Available online 20 August 2004

**Abstract**—The discovery of a series of quinazolinone-based fungal efflux pump inhibitors by high-throughput screening for potentiation of fluconazole in *C. albicans* is described. Attempts to improve the aqueous solubility of screening hits led to the discovery of an analog with greatly improved physical properties and activity against clinically-relevant *Candida* spp.


## 1. Introduction

In recent years there has been a marked increase in the incidence of systemic fungal infections due to organisms that are not susceptible to commonly-used antifungal agents such as fluconazole.[1] Intrinsic and acquired resistance to azoles in *Candida* spp.[2] and *Aspergillus fumigatus*[3] has been associated with the overproduction of multidrug transporters of the ABC and Major Facilitator superfamilies. The precedent for the potentiation of antimicrobial agents through the inhibition of efflux pumps in bacteria by a variety of molecular classes is now well established,[4] and the potential for the analogous potentiation of antifungal agents through combination with fungal efflux pump inhibitors is, therefore, readily apparent.

Recently, we reported the isolation and characterization of the first fungal efflux pump inhibitors (FEPIs).[5] The molecules were identified in a high-throughput screen for potentiation of fluconazole versus *C. albicans*, and were subsequently validated as fungal efflux pump inhibitors using mode of action assays designed in these laboratories. [6] Several structurally-diverse classes of hits were obtained, some of which inhibited pumps from more than one family simultaneously. However, our attention was drawn to a series of quinazolinones which, although apparently specific for inhibition of CDR1 in *C. albicans*, represented attractive starting points for medicinal chemistry due to their potency and their relative ease of synthesis.

Two representative hits (**1**, **2**) are illustrated in Figure 1, and their activity in vitro is summarized in Table 1. The fluconazole potentiation activity is conveniently expressed as the lowest concentration of FEPI achieving an 8-fold reduction in fluconazole MIC (Minimum

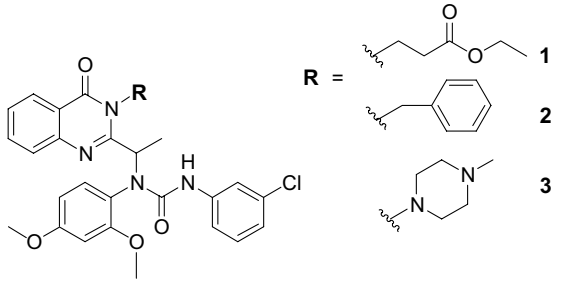



**Figure 1.** Structures of **1**, **2**, and **3**.

* Corresponding author at present address: Inflammatory and Viral Diseases Unit, Roche Palo Alto, 3431 Hillview Avenue, R6E-3, Palo Alto, CA 94304, USA. Tel.: +1 650 855 5774; fax: +1 650 852 1311; e-mail: remy.lemoine@roche.com
† E-mail: wwatkins@gilead.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.07.070

**Table 1.** In vitro activity of two representative screening hits

| No. | Concn required for 50% pump inhibition in *S. cerevisiae* (μg/mL)[a] | | | | $MPC_8$ (μg/mL) | |
|---|---|---|---|---|---|---|
| | CDR1 | CDR2 | CgCDR1 | CgCDR2 | *C. albicans* | *C. glabrata* |
| **1** | 0.5 | >32 | >32 | >32 | 1 | >32 |
| **2** | 0.125 | >32 | >32 | >32 | 0.5 | >32 |

[a] See Materials and methods.

Potentiation Concentration, or $MPC_8$). The spectrum of pump inhibition of each compound is also characterized using isogenic strains of *Saccharomyces cerevisiae* (derived from YKKB-13) in which PDR5 is deleted and individual CDR pumps are over-expressed under the control of a common promoter.[7]

In this paper, we describe the discovery of **3**, the first example of the series with fluconazole potentiation activity against a wide range of clinically relevant *Candida* spp. and with dramatically improved aqueous solubility.

New compounds were initially synthesized as racemates according to the general synthetic scheme shown in Scheme 1.

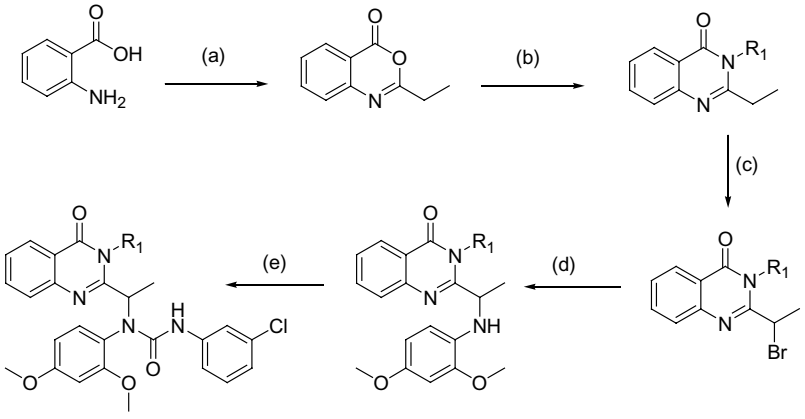
The quinazolinone bicyclic system was readily derived from anthranilic acid, via the intermediacy of the isatoic anhydride formed by condensation with propionic anhydride;[8] this route was particularly conducive to facile variation at $R_1$. Following bromination, displacement by the relatively nucleophilic 2,4-dimethoxyaniline was achieved under mild conditions. Finally, reaction with 3-chlorophenylisocyanate introduced the urea functionality.

At room temperature, the proton NMR spectra of final products were more complex than expected. However, we showed, that at 100 °C, all the protons gave discrete and readily-interpretable signals. We suggest that at room temperature the quinazolinones are composed of a set of conformational isomers that interconvert on the NMR time scale. This set could include atropodiastereoisomers since atropoisomerism has already been reported in quinazolinones.[9]

The limited aqueous solubility of **1** and **2** (<1 μg/mL at pH = 7) was a cause for concern. As these analogs differ only at $R_1$, we explored the introduction of ionizable groups at this position. Gratifyingly, the solubility of the piperazine-containing compound **3** (see Fig. 1) was dramatically improved (130 μg/mL at pH = 7; 2 mg/mL in water as the mesylate salt).

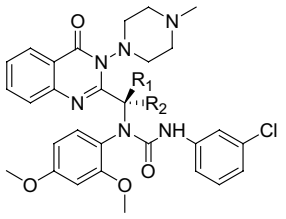
Quite unexpectedly, **3** displayed a second important advantage: it inhibited pumps in both *C. glabrata* as well as *C. albicans* (Table 2). Subsequent studies showed that **3** also potentiates the activity of FLU versus *C. tropicalis*, *C. parapsilosis* and, to a lesser extent, *C. krusei*, although no activity was seen versus strains of *S. cerevisiae* over-expressing efflux pumps other than from the ABC superfamily (data not shown). The broader spectrum of activity of **3** was of great significance to the program, given the prevalence of these species in fluconazole-resistant yeast infections. Other analogs bearing an *N*-methylpiperazinyl substituent on any of the aryl rings rather than at $R_1$ lacked activity versus *C. glabrata*, indicating that the basic nitrogen at $R_1$ was critical for the broad-spectrum activity of **3**.

Given the presence of an asymmetric center in **3**, and noting the spectral evidence for rotational barriers, we systematically explored the influence of methyl substituents at this position upon activity. The enantiomers of **3** were ultimately derived from the requisite lactic acid[10] (Scheme 2).

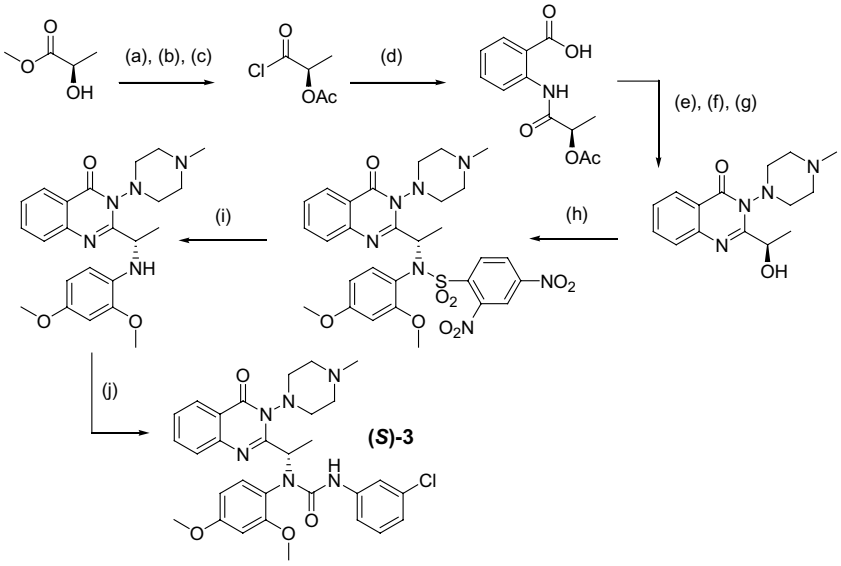



**Scheme 1.** General racemic synthesis. Reagents and conditions: (a) $(EtCO)_2O$, 70 °C; (b) $R_1$-$NH_2$, AcOH, 70 °C; (c) $Br_2$, NaOAc, AcOH, rt; (d) 2,4-dimethoxyaniline, $Na_2CO_3$, DMF, 80 °C; (e) 3-chlorophenylisocyanate, $CH_2Cl_2$, 40 °C.

**Table 2.** In vitro activity of **3**, **4**, **5**, **3**-(***S***), and **3**-(***R***)




| No. | Concn required for 50% pump inhibition in *S. cerevisiae* (μg/mL) | | | | $MPC_8$ (μg/mL) | |
|---|---|---|---|---|---|---|
| | CDR1 | CDR2 | CgCDR1 | CgCDR2 | *C. albicans* | *C. glabrata* |
| **3** | 4 | >16 | 8 | >16 | 1 | 4 |
| (***S***)-**3** | 2 | >32 | 4 | >32 | 1 | 1 |
| (***R***)-**3** | >32 | >32 | >32 | >32 | >32 | >32 |
| **4** | 32 | >32 | >32 | 32 | 16 | 16 |
| **5** | >32 | >32 | >32 | >32 | >32 | >32 |




**Scheme 2.** Homochiral synthesis of (*S*)-**3**. Reagents and conditions: (a) LiOH, MeOH, rt; (b) AcOH, $H_2SO_4$, reflux; (c) $SOCl_2$, reflux; (d) anthranilic acid, aq $NaHCO_3$, AcOEt, 0 °C; (e) $Ac_2O$, 70 °C. (f) 1-amino-4-methyl-piperazine, AcOH, 70 °C; (g) $Et_3N$, MeOH, $H_2O$, rt. (h) *N*-(2,4-dimethoxyphenyl)-(2,4-dinitrobenzenesulfonamide), $PPh_3$, DIAD, THF, rt; (i) $HSCH_2COOH$, $Et_3N$, $CH_2Cl_2$, rt; (j) 3-chlorophenylisocyanate, $CH_2Cl_2$, rt.

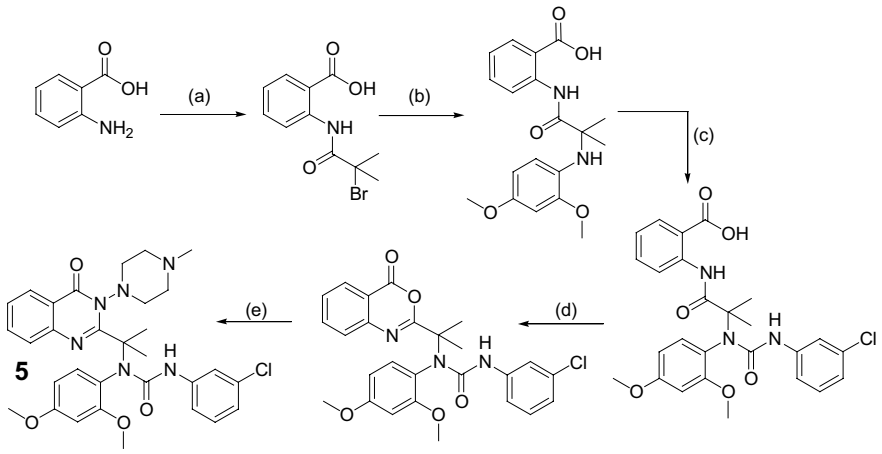
Racemization during the alkylation of the aniline was minimized by resorting to a Mitsunobu-type displacement, and the optical purity of the final product was shown by chiral HPLC to be >95% in each case.

The synthesis of the des-methyl compound **4** was analogous to that of **3**, replacing propionic anhydride with acetic anhydride; the dimethyl analog **5** was made as shown (Scheme 3).

All the activity of **3** is seen to reside in the (*S*) enantiomer, which is markedly more active than any other analog in this set (see Table 2). The proton NMR of **4** at room temperature showed discrete signals indicating a much faster interconversion rate between the conformational isomers (or possible atropoisomers) in **4** as compared to **3**. These data suggest that the role of the methyl substituent on the asymmetric carbon is to serve as a conformational lock, rendering the piperazine and the three aryl residues suitably disposed for interaction with the pump. A global computational search by semi-empirical methods failed to identify a single low-energy conformer for **3**; three discrete sets in which the relative orientations of the aryl groups and the piperazine ring differ significantly were observed within 2 kcal/mol of the global minimum (Fig. 2).

The population of low-energy conformers of **4** and **5** differed markedly from **3**, supporting the notion that the methyl group, while important for activity, may not be in direct contact with the pump.

Attempts to improve the aqueous solubility of screening hits led to the discovery of **3**, the first quinazolinone-based




**Scheme 3.** Synthesis of **5**. Reagents and conditions: (a) $BrC(CH_3)_2COCl$, aq $NaHCO_3$, AcOEt, 0 °C; (b) 2,4-dimethoxyaniline, $K_2CO_3$, EtOH, reflux; (c) 3-chlorophenylisocyanate, $CH_2Cl_2$, rt; (d) $Ac_2O$, 80 °C; (e) 1-amino-4-methylpiperazine, AcOH, 70 °C.

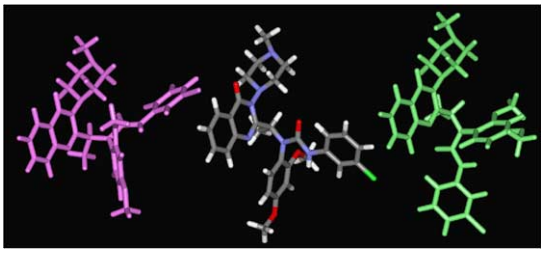


**Figure 2.** Low-energy conformers of **3**. The global minimum is depicted in CPK colors and the two other conformers within 2 kcal/mol in magenta and green.

FEPI showing fluconazole potentiation in clinically relevant *Candida* spp. Investigation into the role of the methyl substituent on the asymmetric center demonstrated that all the activity in **3** resides in the (*S*) enantiomer, and supports the notion that the primary role of the substituent is to rigidify the structure, leaving the piperazine moiety and the three aryl residues suitably disposed for efficient pump inhibition.

In vitro SAR for pump inhibition and optimization of the pharmacokinetic characteristics of the series will be the subject of further publications.[11]

## 2. Materials and methods

### 2.1. Chemicals

All FEPIs described herein were purified by chromatography on silica gel, eluting with varying proportions of ethyl acetate and hexanes or methanol and dichloromethane. The structural identity of each compound was confirmed by $^1H$ NMR and MS. Optical purity of homochiral compounds was measured by chiral chromatography on a Chiralpack AD 250 × 4.6 mm column, eluting with 30% 2-propanol in hexanes at 1 mL/min. Absolute stereochemistry was assigned by analogy with relative retention times of both enantiomers.

### 2.2. Solubility assays

Compounds were suspended in 0.05 M potassium phosphate buffer (pH 7.0), and sonicated for 15 s. After an hour on a shaker, the sample was centrifuged and the amount of compound in solution was quantified by HPLC (using an Agilent Zorbax 4.6 × 150 mm column, eluting with an acetonitrile–water gradient at 0.5 mL/min) against a standard curve. While similar solubility studies were not performed in microbiological medium, no precipitation was observed at the test concentrations employed.

### 2.3. In vitro potentiation of fluconazole

Fluconazole (FLU) MICs in the presence or absence of inhibitors were determined in RPMI using a broth microdilution version of the NCCLS reference method.[12] MICs were measured in YEM 15, a FLU-resistant (MIC = 128 μg/mL) laboratory strain of *Candida albicans* over-expressing the ABC transporters CDR1 and CDR2, and in YEM 19, a strain of *C. glabrata* overexpressing CgCDR1 and CgCDR2. The activity of the inhibitors was quantified by the term $MPC_8$, which is the minimum concentration (μg/mL) of inhibitor required to decrease (potentiate) the MIC of FLU 8-fold. None of the compounds described herein showed any intrinsic antifungal activity. Not all of the analogs reported in this study were assessed in full checkerboard format; an abbreviated method of evaluation that involved testing varying concentrations of efflux pump inhibitor in the presence of fixed concentrations of fluconazole equivalent to 1/8 and 1/32 MIC. This method was validated on a set of eight analogs by comparison with the $MPC_8$ values derived from full checkerboard studies; the results were identical.

The inhibition of specific ABC transporters from *C. albicans* and *C. glabrata* was assessed using defined strains of *S. cerevisiae*: YEM139 (PDR5 deleted; FLU MIC 0.5 μg/mL), YEM172 (CDR1 over-expressed; FLU MIC 128 μg/mL), YEM171 (CDR2 over-expressed;

FLU MIC 8 μg/mL), Y170 (CgCDR1 over-expressed; FLU MIC 128 μg/mL), and Y218 (CgCDR2 over-expressed; FLU MIC 2 μg/mL). An inoculum of $10^4$ cells of each strain was incubated at 30 °C in YPD in the presence of varying concentrations of inhibitor and a single concentration of fluconazole corresponding to the geometric mean of the MIC of the test strain and the MIC of the parent pump-deleted strain (i.e., YEM172 was incubated with 8 μg/ml fluconazole, which is the geometric mean of 128 (MIC of YEM 172) and 0.5 (MIC of YEM167). MICs were determined as 80% inhibition of growth after 48 h.

## References and notes


1. (a) Klepser, M. E. *Pharmacotherapy* **2001**, *21*, 124S; (b) Stevens, D. A.; Holmberg, K. *Curr. Opin. Anti-Infect. Invest. Drugs* **1999**, *1*, 306; (c) Vanden Bossche, H.; Dromer, F.; Improvisi, I.; Lozano-Chiu, M.; Rex, J. H.; Sanglard, D. *Med. Mycol.* **1998**, *36*(Suppl. 1), 119.
2. (a) Katiyar, S. K.; Edlind, T. D. *Med. Mycol.* **2001**, *39*, 109; (b) Barchiesi, F.; Calabrese, D.; Sanglard, D.; di Francesco, L. F.; Caselli, F.; Giannini, D.; Giacometti, A.; Gavaudan, S.; Scalise, G. *Antimicrob. Agents Chemother.* **2000**, *44*, 1578; (c) Sanglard, D.; Ischer, F.; Calabrese, D.; Majcherczyk, P. A.; Bille, J. *Antimicrob. Agents Chemother.* **1999**, *43*, 2753; (d) Sanglard, D.; Kuchler, K.; Ischer, F.; Pagani, J. L.; Monod, M.; Bille, J. *Antimicrob. Agents Chemother.* **1995**, *39*, 2378.
3. Slaven, J. W.; Anderson, M. J.; Sanglard, D.; Dixon, G. K.; Bille, J.; Roberts, I. S.; Denning, D. W. In 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, Sept 1999; Abstract 447.
4. Renau, T. E.; Lemoine, R. C. *Drugs of the Future* **2001**, *26*, 1171.
5. Lomovskaya, O.; Warren, M.; Mistry, A.; Staley, J.; Galazzo, J.; Fuernkranz, H.; Lee, M.; Miller, G.; Sanglard, D. In 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, Sept 1999; Abstract 1269; further publications on the biological aspects of this work will be forthcoming.
6. Mistry, A.; Warren, M. S.; Blais, J.; Staley, A. L.; Galazzo, J. L.; Fuernkranz, H.; Chamberland, S.; Lee, M. D.; Watkins, W. J.; Lomovskaya, O.; Miller, G. H.; Sanglard, D. In 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, ON, Sept 2000; Abstract 1500.
7. The authors are grateful to D. Sanglard for the provision of the plasmids used in this study; see also Ref. 4.
8. Smith, K.; El-Hiti, G. A.; Abdel-Megeed, M. F.; Abdo, M. *J. Org. Chem.* **1996**, *61*, 647, et op. cit.
9. Dai, X.; Wong, A.; Virgil, S. C. *J. Org. Chem.* **1998**, *63*, 2597.
10. Diastereomeric excesses were determined by reaction of the acyl chloride with enantiomerically-pure 2-ethylbenzylamine and analysis of the products using the chiral column described in Materials and Methods.
11. Watkins, W. J.; Lemoine, R. C.; Chong, L.; Cho, A.; Renau, T. E.; Kuo, B.; Wong, V.; Ludwikow, M.; Garizi, N.; Iqbal, N.; Barnard, J.; Jankowska, R.; Singh, R.; Madsen, D.; Lolans, K.; Lomovskaya, O.; Oza, U.; Dudley, M. N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, following paper in this issue. doi:10.1016/j.bmcl.2004.07.071.
12. NCCLS. 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. NCCLS document M27-A.