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Bioorganic & Medicinal Chemistry 12 (2004) 6255–6269
Bioorganic & Medicinal Chemistry

Carboxylic acid and phosphate ester derivatives of fluconazole: synthesis and antifungal activities

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Received 12 July 2004; revised 27 August 2004; accepted 27 August 2004
Available online 21 September 2004

Abstract—Two classes of fluconazole derivatives, (a) carboxylic acid esters and (b) fatty alcohol and carbohydrate phosphate esters, were synthesized and evaluated in vitro against *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus niger*. All carboxylic acid ester derivatives of fluconazole (**1a**–**l**), such as *O*-2-bromooctanoylfluconazole (**1g**, MIC = 111 μg/mL) and *O*-11-bromoundecanoylfluconazole (**1j**, MIC = 198 μg/mL), exhibited higher antifungal activity than fluconazole (MIC ⩾ 4444 μg/mL) against *C. albicans* ATCC 14053 in SDB medium. Several fatty alcohol phosphate triester derivatives of fluconazole, such as **2a**, **2b**, **2f**, **2g**, and **2h**, exhibited enhanced antifungal activities against *C. albicans* and/or *A. niger* compared to fluconazole in SDB medium. For example, 2-cyanoethyl-ω-undecylenyl fluconazole phosphate **(2b)** with MIC value of 122 μg/mL had at least 36 times greater antifungal activity than fluconazole against *C. albicans* in SDB medium. Methyl-undecanyl fluconazole phosphate **(2f)** with a MIC value of 190 μg/mL was at least 3-fold more potent than fluconazole against *A. niger* ATCC 16404. All compounds had higher estimated lipophilicity and dermal permeability than those for fluoconazole. These results demonstrate the potential of these antifungal agents for further development as sustained-release topical antifungal chemotherapeutic agents.

1. Introduction

In recent years fungal infections have emerged as a major cause of disease and mortality, in part as a consequence of the increase in acquired immunodeficiency syndrome (AIDS), the greater use of immunosuppressive drugs in transplantation and chemotherapeutic agents in cancer, long term use of corticosteroids, and even the indiscriminate use of antibiotics.[1–3] Fungal infections are common complications of infection with human immunodeficiency virus (HIV). Over 90% of those diagnosed to be HIV-positive contract a fungal infection during the course of their illness.[4,5] *Cryptococcus neoformans* is the major cause of meningitis in AIDS patients, and it can also cause local organ dysfunction and disseminated disease.[6] In the case of transplantation, the mortality directly attributable to fungal infections carries exceedingly high costs mainly as loss of the organ transplant and the patient.

There are effective antifungal agents in the market, but each drug carries several drawbacks. The presently marketed antifungal drugs are either highly toxic (e.g., amphotericin B, AMB) or are becoming ineffective due to appearance of resistant strains (e.g., flucytosine and azoles). AMB remains the 'gold standard' drug for life-threatening fungal infections, but its use is limited due to its severe toxicity and the inconvenience of intravenous dosing.[7,8] Fluconazole (FLC) is an orally effective azole-based antifungal drug with low toxicity,[9] but it has a limited antifungal spectrum and is not fungicidal. Although FLC shows a very significant efficacy against *Candida albicans* and *C. neoformans*, it is not very effective against *Aspergillus niger*[10,11] and *Aspergillus fumigatus*.[12] In addition, extensive use of FLC has increased the number of FLC-resistant *C. albicans* isolates.[13] Therefore, toxicity concerns, limited spectrum, and the emergence of fungi resistant to currently available agents has created a need for new and effective antifungal agents against life-threatening systemic mycoses caused by *C. neoformans*, *Aspergillus* species, and *Candida* species more urgent. These efforts led to discovery of several azole and triazole derivatives (e.g., itraconazole and voriconazole)[14] as well as several

Keywords: Antifungal activity; Synthesis; Carboxylic acid esters; Phosphate esters.
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doi:10.1016/j.bmc.2004.08.049

(1→3)-β-D-glucan synthase inhibitors (e.g., capsofungin, echinocandine).[15–17] Voriconazole has excellent activity against *Aspergillus* and is generally well tolerated. Caspofungin has recently been shown to offer an excellent alternative to AMB (with less toxicity) or FLC (with a broader spectrum) for therapy of systemic *Candida* infections.[15–17]

In search for new agents with improved antifungal profiles, we have designed two classes of FLC derivatives, (a) carboxylic acid esters and (b) fatty alcohol and carbohydrate phosphate diesters and triesters (Fig. 1) for potential topical application. Drugs applied to the skin are poorly absorbed. Drug absorption from skin can be improved by increasing lipophilicity. A sustained release of FLC from conjugate esters could be beneficial in treatment of superficial fungal infections. This could result in a requirement for less frequent dosages of the conjugate ester analog compared to those of the conjugate ester components alone. The rationale for this drug design approach was based on the expectation that the carboxylic acid ester, phosphate diester, or phosphate triester derivatives of FLC will enhance the lipophilicity of FLC and will be slowly biotransformed through hydrolysis and/or enzymatic cleavage to FLC and carboxylic acid, fatty alcohol, or carbohydrate analogs. Additionally, a number of selected carboxylic acids and fatty alcohols possess antifungal activities. Each individual component could exhibit antifungal activity resulting in enhanced and/or broad-spectrum antifungal activities. Three opportunistic fungal organisms, *A. niger*, *C. neoformans*, and *C. albicans*, were used in all antifungal tests.

Carboxylic acid-FLC or fatty alcohol phosphate-FLC conjugates may allow a larger concentration of intact conjugate ester to enter the infected cells, due to the lipophilic nature of the fatty acids or the fatty alcohols. In addition to the possibility of showing enhanced lipophilicity, potency, and/or broad-spectrum antifungal activities, the carboxylic acid-FLC or fatty alcohol phosphate-FLC conjugates may have additional advantages. The development of fungal resistance to those conjugates, which have two active components acting on different targets, would be less likely than to either compound alone since the two components of conjugate ester could conceivably act by different mechanisms.

FLC-carboxylic acid ester conjugates (**1a**–**l**) (Scheme 1) were designed to increase the lipophilicity of FLC and with the expectation that two components of the compounds will be slowly released upon ester cleavage. FLC prevents the synthesis of ergosterol, a major component of fungal plasma membranes, by inhibiting the cytochrome P-450-dependent enzyme lanosterol demethylase (also referred to as 14α-sterol demethylase or P-450$_{DM}$).[18,19]

A number of fatty acids exhibit antifungal activity by different mechanisms.[20–24] However, it is proposed that

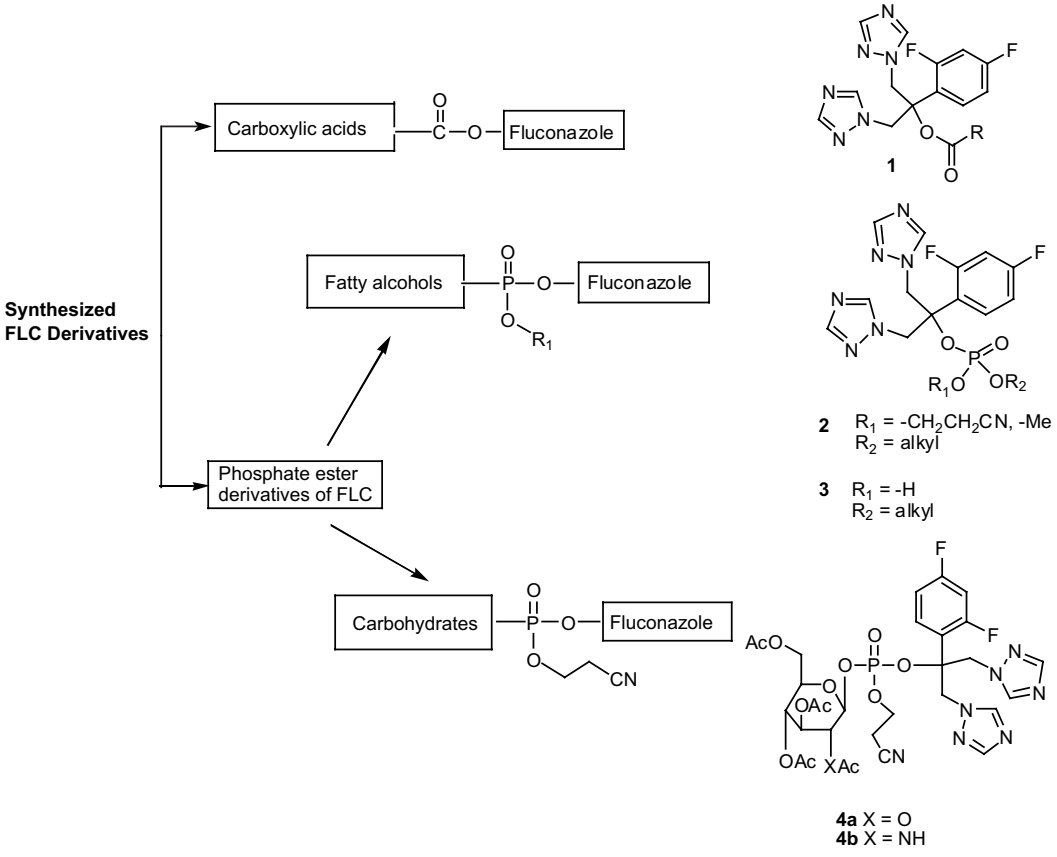


Figure 1. Two classes of synthesized FLC derivatives.

they act by a combination of mechanisms. Fatty acids, in general, have been reported to inhibit cellular respiration,[25] phosphoceramide synthase of the sphingolipid biosynthetic pathway,[26] and fungal precursor protein myristoylation or more specifically myristoyl-CoA: protein *N*-myristoyl transferase (NMT).[27] The partial incorporation of fatty acids through membrane lipid biosynthesis may account for their differential activity.[28] For example, studies with 4-oxatetradecanoic acid analogs have already indicated that they enter fungal cells and are converted to their CoA thioesters by cellular acylCoA synthetase, and are delivered to target proteins by NMT. Many fungal species like *Saccharomyces cerevisiae*, *C. albicans*, and *C. neoformans* synthesize a variety of *N*-myristoyl proteins including ADP ribosylation factor (Arf1p, Arf2p) using NMT as catalyst.[27,29,30] In several cases their mechanism of antifungal activity is not known. The 2-substituted tetradecanoic acids were reported to be active against *C. neoformans*, *A. niger*, *C. albicans*, and *S. cerevisiae*.[21] Thus, a number of 2-bromosubstituted fatty acyl ester analogs of FLC were synthesized to determine their antifungal properties.

Fatty alcohol derivatives such as pramanicin,[31] lipoxamycin,[32] and neoenactin A[33–35] have been shown to have promising antifungal activities. A series of aliphatic alkanols have been reported to exhibit significant antifungal activity.[36] In this work we selected several fatty alcohols and conjugated them with FLC through a phosphate linker. It was expected that resulting double-barreled compounds, fatty alcohol phosphate triester (**2a**–**h**) and phosphate diester (**3a**–**d**) derivatives of FLC (Scheme 2), to have enhanced antifungal activity compared to those of carboxylic acid ester derivatives of FLC (**1a**–**l**) and FLC.

Several carbohydrates and carbohydrates derivatives such as CAN-296,[37,38] derivatives of 2-amino-2-deoxy-D-glucitol-6-phosphate,[39] zargozic acid and its derivatives,[40,41] papulacandins such as papulacandin B,[42] and carbohydrates with structures similar to inositol,[43] have been reported to have antifungal activities by a variety of mechanisms. Many fungi possess specific carbohydrates integrated in the structure of their cell wall.[44–46] The absence of some of these specific carbohydrates in the structure of mammalian cells may create an opportunity to design conjugate ester analogs, which may selectively inhibit fungal cells. For the purpose of this research, two phosphate triester glucose derivatives of FLC (**4a** and **4b**) (Scheme 3) were synthesized and their antifungal activities were compared with those of the fatty alcohol phosphate triesters derivatives of FLC (**2a**–**h**).

2. Results and discussion

2.1. Chemistry

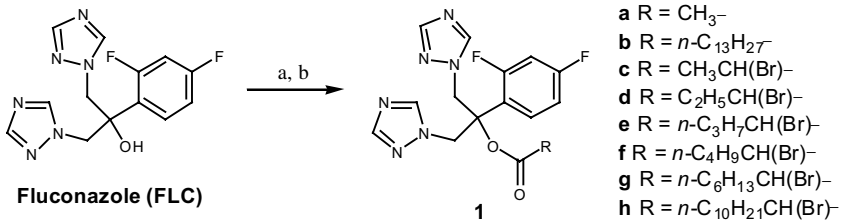
Scheme 1 shows the procedure for the preparation of carboxylic acid ester derivatives of FLC (**1a**–**h**) from the reaction of acyl chlorides with FLC in the presence of sodium hydride. Acyl chlorides were synthesized from the corresponding carboxylic acids with thionyl chloride in dry benzene if they were not commercially available.

Scheme 2 demonstrates the preparation of phosphate triester derivatives of FLC (**2a**–**h**). The reaction of alcohol (ROH) with phosphorylating reagents, cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, or methyl *N*,*N*-diisopropylchlorophosphoramidite **(5)**, in the presence of diisopropyl ethylamine in dry DCM gave *N*,*N*-diisopropylamine phosphine derivatives **(6)**. Coupling reactions of FLC with **6** in the presence of 1-*H*-tetrazole in DCM followed by oxidation with *t*-butyl hydroperoxide afforded phosphate triesters (**2a**–**h**). The cyanoethyl protecting group in **2a**–**d** was cleaved using ammonium hydroxide to yield the corresponding phosphate diester derivatives of FLC (**3a**–**d**).

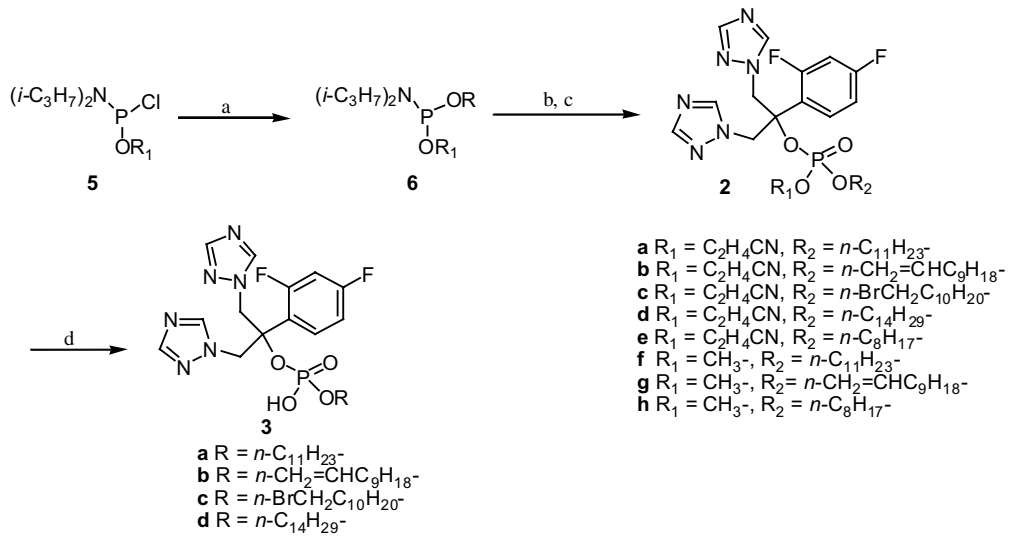
Scheme 3 displays the synthesis of carbohydrate phosphate triester derivatives of FLC (**4a** and **4b**). The reaction of β-D-glucosepentaacetate **(7a)** or 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**7b**) with benzylamine in THF resulted in the selective removal of the anomeric *O*-acetyl group to produce the reducing carbohydrates **8a** and **8b**. Reaction of **8** with cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite followed by coupling with FLC and oxidation reaction in the presence of *t*-butyl hydroperoxide furnished carbohydrate phosphate triesters (**4a** and **4b**).

2.2. Antifungal activity

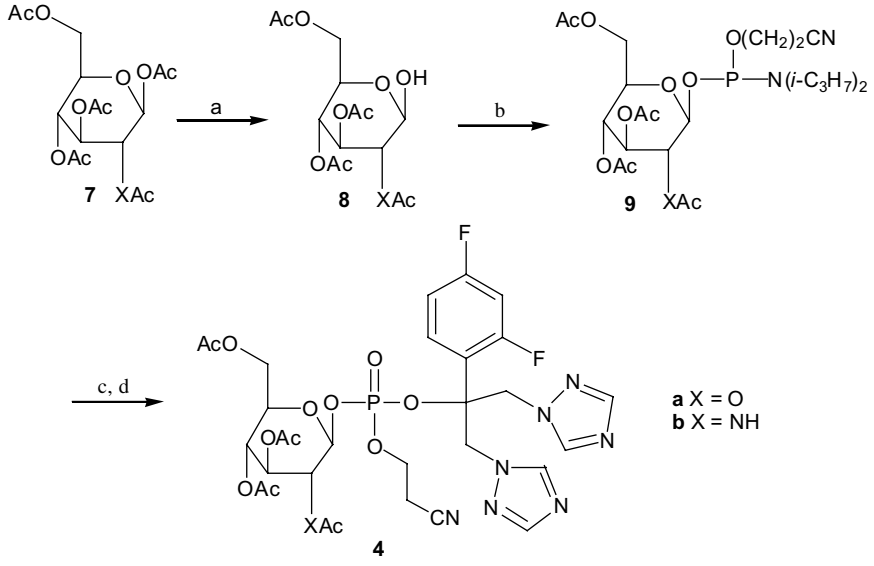
Biological data for FLC derivatives show that the in vitro antifungal activity is clearly dependent upon the



Scheme 1. Synthesis of carboxylic acid ester derivatives of FLC. Reagents and conditions: (a) NaH, DMF, rt, 2 h; (b) RCOCl, rt, 3 h.



Scheme 2. Synthesis of alkylphosphate derivatives of FLC. Reagents and conditions: (a) ROH, DIEA, DCM, rt, 20 h; (b) FLC, 1*H*-tetrazole, DCM, 2 h, rt; (c) *t*BuOOH (28%), 0 °C, 1 h; (d) NH_4OH/MeOH, 24 h, rt.

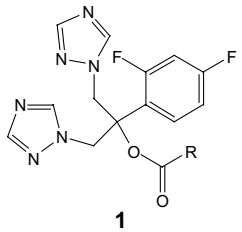


Scheme 3. Synthesis of carbohydrate phosphate triester derivatives of FLC. Reagents and conditions: (a) $BnNH_2$, THF; (b) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIEA, DCM, 20 h; (c) 1. FLC, 1*H*-tetrazole, DCM, 2 h, rt; (d) *t*BuOOH, 0 °C, 1 h.

nature of substituent(s) attached to FLC and/or incorporated into the chain of carboxylic acids or fatty alcohols. These groups of compounds exhibited a broad range of antifungal activities.

2.2.1. Carboxylic acid ester derivatives of FLC (1). The in vitro antifungal test results for carboxylic acid ester derivatives of FLC (**1**) are presented in Table 1. FLC was active against *C. albicans* in RPMI medium (MIC = 2.3 μg/mL), but showed complete resistance against *C. albicans* in SDB medium (MIC ⩾ 4444 μg/mL). All carboxylic acid derivatives of FLC (**1a**–**l**) exhibited lower antifungal activity than FLC in RPMI 1640 growth medium. Two compounds, *O*-2-bromooctanoylfluconazole (**1g**) (MIC ⩽ 14 μg/mL) and *O*-11-bromoundecanoylfluconazole (**1j**, MIC = 12 μg/mL) exhibited antifungal activity against *C. albicans* in RPMI and were the most active antifungal agents in this group of compounds against all fungi tested. Since these compounds are carboxylic esters, it was expected that they could be partially or completely hydrolyzed in vitro, releasing FLC and carboxylic acids. Therefore, the true activity of these compounds may not be determined accurately in vitro RPMI medium with pH = 6.9. The half-life for chemical hydrolysis of all 2-bromosubstituted ester derivatives of FLC (**1a**–**i**) was calculated to be approximately 74 days at pH 6.9 using the HYDROWIN (Version 1.67) estimation program. The hydrolysis rate is slow probably due to the steric hindrance around the ester functional group, surrounded by three aromatic groups and long chain alkyl chain, sometimes substituted with bulky substituents such as bromine. On the

Table 1. General structure, physicochemical properties, and antifungal activity (MIC values, μg/mL)[a] against *Candida albicans* (RPMI and SDB), *Cryptococcus neoformans* (SDB), and *Aspergillus niger* (SDB) at 35–37 °C after 24–48 h and for carboxylic acid ester derivatives of FLC (**1**)



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Compound	MIC (μg/mL)				Log P[b]	K_p (μ/h)[c]
	C. albicans ATCC 14053, RPMI	*C. albicans* ATCC 14053, SDB	*C. neoformans* ATCC 66031, SDB	*A. niger* ATCC 16404, SDB		
1a	>1420	1420	>1420	⩾1420	1.3	1.1
1b	718	718	718	⩾718	7.2	1610
1c	656	656	656	>656	2.0	1.1
1d	319	636	>1276	⩾1276	2.5	1.9
1e	275	552	1100	⩾1100	3.0	3.5
1f	61	492	492	⩾979	3.5	6.5
1g	⩽14	111	14	111	4.5	21.8
1h	1466	1466	1466	⩾1466	6.4	246
1i	731	731	731	>731	7.4	826
1j	12	198	6.1	395	6.0	151
1k	977	>977	>977	>977	1.9	1.2
1l	⩾1212	>1212	>1212	>1212	2.3	1.2
FLC	2.3	⩾4444	2.4	>580	0.3	0.4
AMB	0.7	1.5	<3.7	0.4	−3.8	5.1×10^{-8}
DMSO	>5000	>5000	>5000	>5000		

[a] The result is the average of three separate experiments.
[b] Partition coefficient of the compound calculated using the KowWin program.
[c] Dermal permeability coefficient of the compound calculated using the DermWin program (μ/h).

other hand, all compounds showed higher antifungal activity than FLC against *C. albicans* in SDB medium (pH = 5.6). FLC was completely inactive against *C. albicans* in SDB suggesting that the antifungal effects of carboxylic ester derivatives of FLC (**1a**–**l**) are not exclusively due to ester hydrolysis and the release of FLC. Compounds **1g** (MIC = 111 μg/mL) and **1j** (MIC = 198 μg/mL) were at least 40- and 22-fold more active against *C. albicans* in SDB, respectively, than that for FLC. Furthermore, compounds **1g** (MIC = 111 μg/mL) and **1j** (MIC = 395 μg/mL) were at least 5.2- and 1.5-fold more potent than FLC against *A. niger* indicating the importance role of carboxylic acids attached to FLC through the ester moiety in enhancing antifungal activity. The antifungal activity of carboxylic ester **1j** against *C. neoformans* was comparable to FLC. Although compounds **1g** and **1j** exhibited 8- and 17-fold less antifungal activity against *C. albicans* in SDB than that in RPMI, respectively, they were still significantly more active than FLC and other analogs in SDB, indicating the presence of an additional mechanism(s) other than ester hydrolysis for antifungal activity of these compounds.

Estimated partition coefficients (log P), which can be used as an indicator of passive diffusion across cell membranes and cellular uptake, were determined for all carboxylic ester derivatives of FLC (**1a**–**l**). Increasing the length of ester chain or attaching lipophilic substituents such as bromine, affected lipophilicity dramatically (Table 1). The differences in lipophilicity and the size of the alkyl ester substituent in carboxylic acid ester derivatives of FLC could provide a correlation of antifungal activity with physicochemical properties. All compounds (**1a**–**l**) showed larger estimated partition coefficients (log P = 1.3–7.4) than that for FLT (log P = 0.3). Similarly all carboxylic ester derivatives of FLC (**1a**–**l**) had higher estimated dermal permeability coefficients (K_p) (1.1–1610 μ/h) than that for FLC (K_p = 0.4 μ/h) indicating the potential for using carboxylic ester analogs for dermal delivery of FLC. Since all carboxylic ester derivatives of FLC are lipophlic, their penetration through the lipid matrix of the stratum corneum of skin and also their penetration into cells might be more efficient. More research is required to confirm this postulation. There appears to be a relationship between antifungal activity against all fungi tested and the calculated log P for these carboxylic ester derivatives of FLC. There is a correlation between the number of methylene groups and the antifungal activity of 2-bromosubstituted ester derivatives of FLC (**1c**–**i**). As the number of carbon atoms increases, the antifungal activities are increased up to the maximum level in *O*-2-bromooctanoylfluconazole (**1g**). The antifungal activities are then diminished significantly in compounds **1h** and **1i**, indicating the limit of tolerance for hydrophobicity in generating maximum antifungal activity in this group of compounds. These data suggest that there is an optimal partition coefficient

range for maximum antifungal activity. The conjugate ester *O*-2-bromooctanoylfluconazole (**1g**) has an estimated log*P* value of 4.5 that was optimal among 2-bromosubstituted ester derivatives of FLC (**1c–i**) for maximum antifungal activity. The highly lipophilic compounds, such as **1h** and **1i**, exhibited weaker antifungal activity than other analogs. 2-Bromosubstituted ester analogs with shorter chains, such as **1c–f** or carboxylic acid ester derivatives without a bromine group, such as **1k** and **1l**, generally exhibited weak antifungal activity. Another potent antifungal compound, *O*-11-bromoundecanoylfluconazole (**1j**), has a log*P* value of 6.0 suggesting that additional factors such as the intracellular rate of ester hydrolysis, the stability of ester prior to membrane penetration or in culture medium, and the nature, size, and position of the substituent attached to the chain, may also contribute to overall antifungal effect observed. Differences in the ester hydrolysis are expected to be dependent upon the steric size of the attached acyl group where more sterically hindered esters should possess longer half-lives.

In general 2-bromosubstituted analogs are expected to be much more stable than other carboxylic esters as previously shown in 5′-*O*-myristoyl analog derivatives of zidovudine.[47] Stability of fatty acid derivatives were estimated using HYRDROWIN (Version 1.67) at pH 5.6 as used in SDB medium indicating high stability of these compounds probably due to the presence of a tertiary ester functional group surrounded by bulky aromatic and nonaromatic groups. For example the estimated half-life for all 2-bromosubstituted carboxylic acid esters at pH 5.6 is approximately 4 years. Therefore, chemical hydrolysis does not probably contribute significantly to release of components. It remains to be seen whether the esters are hydrolyzed in vitro by the action of fungal esterases. Further stability studies are underway to determine the stability of these compounds in the presence of fungi and SDB medium and to identify the active component(s) after incubation.

2.2.2. Carboxylic acid and fatty alcohol derivatives. The in vitro antifungal results for several carboxylic acids and fatty alcohols are presented in Table 2. In general, fatty alcohols showed significantly higher antifungal activities against *C. neoformans* and *A. niger* than those of the corresponding fatty acids. For example, tetradecanol (MIC = 14 μg/mL) and 11-bromoundecanol (MIC = 12 μg/mL) were more potent than tetradecanoic acid (MIC > 529 μg/mL) and 11-bromoundecanoic acid (MIC = 23 μg/mL), against *C. neoformans* in SDB, respectively. The antifungal activities of primary aliphatic alcohols from C_6 to C_{13} have been previously reported against *S. cerevisae*.[36] It was proposed that the primary mechanism for the antifungal action of alkanols is due to their amphipathic characteristics and ability as nonionic surfactants to disrupt the native membrane-associated function of the integral proteins.[36] The reason for the higher antifungal activity of the fatty alcohols compared to the corresponding carboxylic acids with similar chain length is not known, but could be due to their better surfactant effect or other mechanism(s) of antifungal action.

Table 2. Physicochemical properties and antifungal activity (MIC values, μg/mL)[a] against *Candida albicans* (RPMI and SDB), *Cryptococcus neoformans* (SDB), and *Aspergillus niger* (SDB) at 35–37 °C after 24–48 h and for carboxylic acid and fatty alcohols

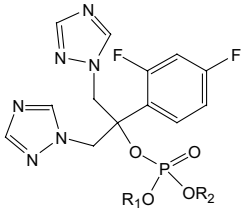
Compound	MIC (μg/mL)				Log *P*[b]	K_p (μ/h)[c]
	C. albicans ATCC 14053, RPMI	*C. albicans* ATCC 14053, SDB	*C. neoformans* ATCC 66031, SDB	*A. niger* ATCC 16404, SDB		
Acetic acid	⩾220	⩾220	⩾220	⩾220	0.1[d]	6.2
2-Bromopropionyl chloride	468	468	117	468	0.3	2.8
2-Bromobutyric acid	513	>513	>513	ND[e]	1.3[f]	18.6
2-Bromovaleric acid	⩾578	578	290	⩾578	1.8	29.9
2-Bromohexanoic acid	⩾710	710	355	710	2.3	54.7
2-Bromooctanoic acid	865	865	433	ND	3.3	184
2-Bromolauric acid	155	155	19	78	5.3	2070
2-Bromomyristic acid	12	>540	15	68	6.3	6970
11-Bromoundecanoic acid	371	185	23	93	4.9	1280
Decanoic acid	791	99	ND	<12	4.0[g]	1360
Tetradecanoic acid	>876	1750	>529	>529	6.0[h]	16,800
1-Undecanol	ND	87	28	87	4.3	1850
ω-Undecylenyl alcohol	ND	110	14	27	4.1	1520
Tetradecanol	ND	1720	14	27	5.8[i]	17,900
11-Bromoundecanol	ND	95	12	24	4.6	1070
FLC	2.3	⩾4444	2.4	>580	0.3	0.4
AMB	0.7	1.5	<3.7	0.4	−3.8	5.1×10^{-8}
DMSO	>5000	>5000	>5000	>5000		

[a] The result is the average of three separate experiments.
[b] Partition coefficient of the compound calculated using the KowWin program.
[c] Dermal permeability coefficient of the compound calculated using the DermWin program (μ/h).
[d] Experimental log*P* = −0.17.[52]
[e] ND = Not determined.
[f] Experimental log*P* = 1.42.[52]
[g] Experimental log*P* = 4.09.[52]
[h] Experimental log*P* = 6.11.[53]
[i] Experimental log*P* = 6.03.[54]

All carboxylic acids were equally active against *C. albicans* in SDB and RPMI media with the exception of 2-bromomyristic acid and 11-bromoundecanoic acid, which showed higher and lower antifungal activity in RPMI, respectively, than that in SDB. 2-Bromolauric, 2-bromomyristic, and 11-bromoundecanoic acids were found to be the most active antifungal agents in this group of carboxylic acids against all fungi tested. 11-Bromoundecanoic acid showed comparable activity to 2-bromolauric acid against all three fungi tested in SDB medium. The antifungal activity of 2-bromomyristic acid and 11-bromoundecanoic acid against *C. albicans* in RPMI medium was in agreement with our earlier studies.[21] There appears to be a relationship between antifungal activity against all fungi tested and the calculated log *P* for 2-bromosubstituted analogs. These data suggest that there is an optimal partition coefficient range for maximum antifungal activity for 2-bromosubstituted carboxylic acids. A log *P* value in the range of 5.3–6.3 is required for the broad-spectrum antifungal activity against fungi tested in SDB medium. 2-Bromosubstituted analogs with shorter chain fatty acids or carboxylic acids without a bromine group generally exhibited weak antifungal activity suggesting the importance of hydrophobicity in the activity of fatty acid derivatives. The cutoff in the observed antifungal activity could be due to a corresponding limit in the absorption of carboxylic acids into lipid-bilayer portions of membranes. Shorter chain carboxylic acids probably enter the cell by passive diffusion across plasma membrane and do not incorporate into the cell membrane. Longer chain and nonbrominated carboxylic acids are probably soluble in the membrane phospholipids, and therefore are incorporated into hydrophobic domain of the membrane. It seems that only amphipathic medium-chain carboxylic acids act as surfactants. All carboxylic acids and fatty alcohols had higher estimated dermal permeability coefficient (K_p = 2.8–17,900 μ/h) than FLC (K_p = 0.39 μ/h).

2.2.3. Phosphate ester derivatives of FLC (2–4). Based on the antifungal activity data for compounds in Table 2, the fatty alcohols were selected for further preparation of phosphate diester and triester derivatives of FLC. The in vitro antifungal test results for the fatty alcohol phosphate triester (**2a**–**h**) and diester (**3a**–**d**) derivatives of FLC are presented in Table 3. In general, in comparison to FLC, this group of compounds had a much stronger activity against *C. albicans* in SDB medium in which FLC was inactive indicating the importance of fatty alcohol portion and phosphate linker in enhancing antifungal activities. FLC phosphate triesters of FLC (**2**) were more potent antifungal agents than phosphate diesters (**3**). Compounds **2a**, **2b**, and **2g** were the most potent antifungal agents in this group against *C. albicans* in SDB medium. Compounds **2a**, **2c**, and **2f** were found to be potent antifungal agents against *C. neoformans* (MIC = 12–31 μg/mL). Compound **2f** with a MIC

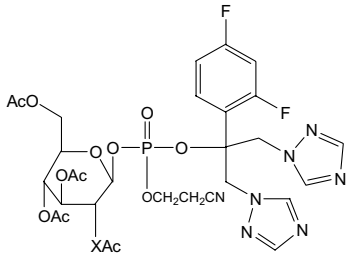
Table 3. General structure, physicochemical properties, and antifungal activity (MIC values, μg/mL)[a] against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus niger* in SDB at 35–37 °C after 24–48 h and for fatty alcohol phosphate diester (**3**) and triester (**2**) derivatives of FLC and controls



Compound	R_1	R_2	MIC (μg/mL)			Log P[b]	K_p (μ/h)[c]
			C. albicans ATCC 14053	*C. neoformans* ATCC 66031	*A. niger* ATCC 16404		
2a	$CNCH_2CH_2$–	*n*-$C_{11}H_{23}$–	185	23	740	4.7	10
2b	$CNCH_2CH_2$–	*n*-CH_2=CH–C_9H_{18}–	122	31	980	4.6	8.3
2c	$CNCH_2CH_2$–	*n*-$BrCH_2C_{10}H_{20}$–	1658	26	1658	5.1	5.8
2d	$CNCH_2CH_2$–	*n*-$C_{14}H_{29}$–	940	235	940	6.2	61.9
2e	$CNCH_2CH_2$–	*n*-C_8H_{17}–	680	170	1360	3.2	1.6
2f	CH_3–	*n*-$C_{11}H_{23}$–	762	12	190	5.2	38.5
2g	CH_3–	*n*-CH_2=CH–C_9H_{18}–	228	29	1825	5.1	31.7
2h	CH_3	*n*-C_8H_{17}–	470	59	470	3.7	6.3
3a	H	*n*-$C_{11}H_{23}$–	760	95	760	5.1	42.8
3b	H	*n*-CH_2=CH–C_9H_{18}–	1501	1501	1501	5.0	35.2
3c	H	*n*-$BrCH_2C_{10}H_{20}$–	1477	185	1477	5.5	24.8
3d	H	*n*-$C_{14}H_{29}$–	940	235	940	6.6	264
FLC			⩾4444	2.4	>580	0.3	0.4
AMB			1.5	<3.7	0.4	−3.8	5.1×10^{-8}
DMSO			>5000	5000	>5000		

[a] The result is the average of three separate experiments.
[b] Partition coefficient of the compound calculated using the KowWin program.
[c] Dermal permeability coefficient of the compound calculated using the DermWin program (μ/h).

Table 4. General structure, physicochemical properties, and antifungal activity (MIC values, μg/mL)[a] against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus niger* in SDB at 35–37 °C after 24–48 h and for carbohydrate phosphate triester derivatives of FLC (**4**) and controls



Compound	MIC (μg/mL)			Log P[b]	K_p (μ/h)[c]
	C. albicans ATCC 14053	*C. neoformans* ATCC 66031	*A. niger* ATCC 16404		
4a	1999	551	1999	−1.3	4.4×10^{-5}
4b	>3399	306	1699	−0.8	1.1×10^{-4}
FLC	⩾4444	2.4	>580	0.3	0.4
AMB	1.5	<3.7	0.4	−3.8	5.1×10^{-8}
DMSO	>5000	>5000	>5000		

[a] The result is the average of three separate experiments.
[b] Partition coefficient of the compound calculated using the KowWin program.
[c] Dermal permeability coefficient of the compound calculated using the DermWin program (μ/h).

value of 190 μg/mL was the most potent compound tested in this group against *A. niger*. The in vitro antifungal activity for these compounds could be dependent upon a number of factors, which include: (i) the intrinsic activity of the intact ester derivative; (ii) the rate and extent of cellular uptake; and/or (iii) the rate and extent at which the two substrate moieties FLC and the fatty alcohol analog are released following hydrolysis of the conjugate ester. The mechanism of intrinsic activity of these derivatives could be due to their amphipathic structure. Hydrophilic parts of the molecule such as protonated triazoles might bind with the hydrophilic portion of fungal cell membrane, while the hydrophobic tail may enter into the membrane lipid bilayer of the membrane. Furthermore, other mechanisms such as hydrolysis of the molecule to FLC and fatty alcohols, which have different mechanisms of antifungal action, may be involved. All compounds in this group had higher estimated partition coefficients ($\log P = 3.2$–6.6) and dermal permeability coefficient ($K_p = 1.6$–264 μ/h) than those for FLC ($\log P = 0.3$; $K_p = 0.4$ μ/h). The data suggest that there is an optimal partition coefficient range (4.6–5.2) for maximum antifungal activity of these compounds against *C. neoformans*.

To understand the importance of fatty alcohols in the antifungal activity of **2**, fatty alcohols were replaced with carbohydrates. Table 4 shows the antifungal activities for carbohydrate phosphate triester derivatives of FLC (**4**). The removal of fatty alcohols in the conjugate and substitution with carbohydrates decreased the antifungal activities significantly against all fungi tested. Compounds **4a** ($\log P = -1.3$) and **4b** ($\log P = -0.8$) showed lower estimated partition coefficients than that for FLC ($\log P = 0.3$). The data indicates the significant role of fatty alcohols in antifungal activity of phosphate triester and diester derivatives of FLC.

2.3. Interaction studies

Although many factors may influence the clinical efficacy of antifungal therapy in vivo, a great effort has been directed toward identifying combinations that are synergistic or antagonistic in vitro. These studies were carried out in detecting and determining such interactions in compounds **1g** and **1j** against *C. albicans* in SDB growth medium, in which FLC was inactive (Fig. 2). Fractional inhibitory concentration (FIC) index [$FIC_{index} = FIC_A + FIC_B$] calculation was used

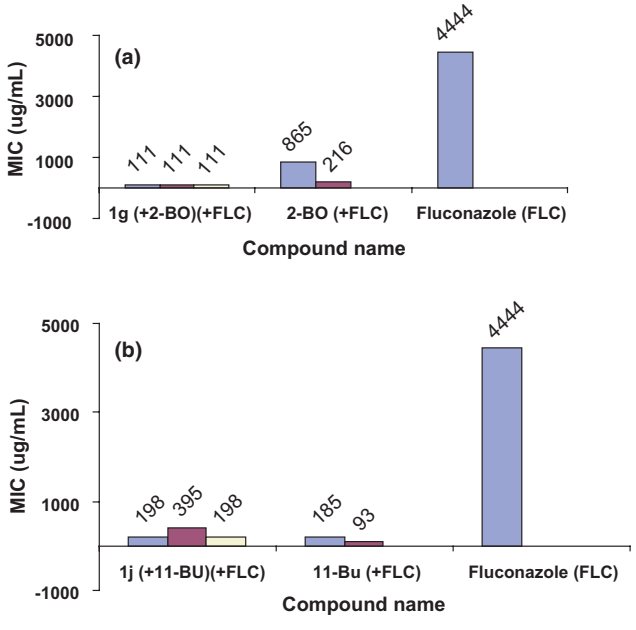


Figure 2. The interaction study of FLC, 2-bromooctanoic acid (2-BO), 11-bromoundecanoic acid (11-Bu) with the conjugate ester antifungal agents, **1g** and **1j**, respectively, against *C. albicans* ATCC 14053 (SDB).

to determine synergism between different components of the synthesized compounds while administered separately, where FIC_A or FIC_B equals to MIC of compounds A or B in combination divided by MIC of drug A or B alone. If the FIC index is $\leqslant 0.5$, the combination is synergistic. If the FIC value is equal to 1, the combination is additive or 'indifferent' and if greater than 4.0, the combination is antagonistic.

While FLC did not show inhibition against *C. albicans* at the highest tested concentration (4444 μg/mL), the mixture of FLC and 2-bromooctanoic (2-BO) showed antifungal activity (Fig. 2a). FIC index values for a mixture of 2-BO and FLC (FIC index = 0.30) indicated a synergism effect between these two components when mixed. There was no synergistic effect when **1g** was mixed with FLC (FIC index = 1.05). The presence of FLC and/or 2-BO did not affect the inhibition level of **1g**. A mixture of 2-BO and FLC had approximately 2-fold less antifungal inhibition than when **1g** was used alone or mixed with any of the components (2-BO or FLC). The inequality in the MIC values of the mixed components and the corresponding conjugate ester **1g** could be due to differences in cell penetration and disposition of individual components compared with the ester derivative of FLC.

Some differences were observed in the interaction studies using 11-bromoundecanoic acid (11-Bu) and **1j** (Fig. 2b). The mixture of 11-Bu with FLC showed synergistic effect (FIC index = 0.52) and fungal inhibitory effect that was approximately equal to that of **1j**. While the presence of FLC did not affect the antifungal activity of **1j** against *C. albicans* in SDB (FIC index = 1.04), addition of 11-Bu reduced the activity of **1j**. The lack of interaction of the designed conjugate esters, **1j** and **1g**, with their individual components (FLC and carboxylic acids), indicate that the ester analogs may have some intrinsic antifungal activity in addition to that of their hydrolysis products, FLC and carboxylic acids, in the site of action.

3. Conclusion

In conclusion, two series of double-barreled carboxylic acid and fatty alcohol derivatives of FLC were designed and their in vitro antifungal structure–activity relationships were determined. By virtue of being prodrugs, all of the synthesized compounds were expected to be less active in vitro in comparison to FLC. Surprisingly, a large difference in antifungal activities was observed for conjugate ester analogs investigated. The advantage of carboxylic acid ester derivatives of FLC is their activity against *C. albicans* ATCC 14053 in SDB medium in which FLC was inactive. The in vitro antifungal activities of several fatty alcohol phosphate diester and triester derivatives of FLC were stronger against *C. albicans* and *A. niger* than those of FLC. Several of these phosphate derivatives were found to be potent antifungal agents against all three fungi indicating their broad-spectrum activities. The exact mode of action of conjugate ester analog derivatives of FLC will obviously remain speculative until further mechanistic investigations have been carried out. Bioconversion studies are underway to determine the stability of candidate compounds following incubation with fungi and SDB medium. Accordingly, in vitro antifungal activity appears to be dependent on several factors, which include intrinsic activity, lipophilicity, rate of cellular uptake, and cellular rate of ester or phosphate ester hydrolysis. The utility of derivatives of FLC as therapeutic agents will be enhanced by a clear understanding of their uptake, metabolism, and incorporation. The in vitro results from this study provide a rationale upon which several candidate compounds can be selected for topical antifungal agents. This strategy can be used for other triazole derivatives such as voriconazole that has a better antifungal profile.

4. Experimental

4.1. General

All products were homogenous by thin-layer chromatography (TLC), performed on Whatman® 250 μm Silica Gel GF Uniplates and visualized under UV light at 254 nm. Melting points were determined with an electrothermal melting point apparatus and are uncorrected. Chromatographic purification was done by the open flash silica gel column chromatography using Merck silica gel 60 (240–400 mesh). Nuclear magnetic resonance spectra (^{1}H NMR) were recorded using tetramethylsilane as an internal standard on a Bruker 400 MHz spectrometer with $CDCl_3$ as a solvent unless otherwise indicated. Chemical shifts are reported in parts per millions (ppm) downfield from tetramethylsilane as an internal standard. Electrospray ionization (ESI) and high-resolution mass spectra were obtained using PE Biosystems API 2000 and Mariner® mass spectrometers, respectively. Reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) unless noted otherwise. Solvents were distilled and dried before use.

4.2. General procedure for the synthesis of carboxylic acid ester derivatives of FLC (1a–h)

Dry dimethyl formamide (DMF) (2 mL) was added to sodium hydride (48 mg, 40% immersion in oil, 1.2 mmol, prewashed with dry hexane) and the mixture was cooled to 0 °C. A solution of FLC (300 mg, 1 mmol) in dry DMF was slowly added to this mixture. After stirring for 2 h at room temperature, the reaction mixture was cooled down again in an ice bath; thereafter a solution of acyl chloride (1.5 mmol, freshly prepared by refluxing the corresponding carboxylic acid with thionyl chloride if not commercially available) in dry benzene was added dropwise during 30 min. The reaction mixture was stirred for a further period of 3 h at room temperature and poured into a separatory funnel containing cold aqueous sodium bicarbonate (5%, 100 mL). The crude product was extracted with ethyl acetate (EA) (2×100 mL). The organic layers were combined, dried

over magnesium sulfate ($MgSO_4$), and concentrated in vacuo. The residue, which consisted of one major product, was purified by a silica gel column chromatography using hexane/acetone (3:1 v/v to 1:1 v/v) as eluting solvents to yield final products (Scheme 1).

4.2.1. *O*-Acetylfluconazole (1a). The general synthetic method described above afforded **1a** (78.0%) as an amorphous powder; mp 131.5–133.1 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97 (2H, s), 7.88 (2H, s), 6.90–6.74 (3H, m), 5.14 (2H, d, J = 14.5 Hz), 5.05 (2H, d, J = 14.5 Hz), 2.15 (3H, s). HR-MS (ESI-TOF) calculated for $C_{16}H_{16}F_2N_6O_2$ 348.1146, found 349.1222 (M+H). ESI m/z 349.2 (M+H), 220.2 (M−$C_4H_5N_3O_2$).

4.2.2. *O*-Tetradecanoylfluconazole (1b). The general synthetic method described above afforded **1b** (67.0%) as an amorphous powder; mp 142–143 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (2H, s), 7.84 (2H, s), 6.88–6.75 (3H, m), 5.15 (2H, d, J = 15.3 Hz), 5.50 (2H, d, J = 15.3 Hz), 2.37 (2H, t, J = 7.5 Hz), 1.54–1.22 (22H, m, methylene envelope), 0.99 (3H, t, J = 8.2 Hz). HR-MS (ESI-TOF) calculated for $C_{28}H_{40}F_2N_6O_2$ 516.3024, found 517.3057 (M+H). ESI m/z 517.3 (M+H), 220.1 (M−$C_{16}H_{29}N_3O_2$).

4.2.3. *O*-2-Bromopropionylfluconazole (1c). The general synthetic method described above afforded **1c** (38.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20 (2H, s), 7.90 (2H, s), 7.08 (1H, d, J = 8.1 Hz), 7.00 (1H, d, J = 2.2 Hz), 6.80 (1H, dd, J = 8.1, 2.2 Hz), 5.39 (2H, d, J = 15.0 Hz), 5.20 (2H, d, J = 15.0 Hz), 4.45 (1H, q, J = 7.5 Hz), 1.83 (3H, d, J = 7.5 Hz). HR-MS (ESI-TOF) calculated for $C_{16}H_{15}BrF_2N_6O_2$ 440.0408, found 441.0411 (M+H, ^{79}Br), 443.1416 (M+H, ^{81}Br). ESI m/z 441.1 (M+H, ^{79}Br), 443.1 (M+H, ^{81}Br), 372.0 (M−$C_2H_2N_3$, ^{79}Br), 374.0 (M−$C_2H_2N_3$, ^{81}Br), 289.3 (M−$C_3H_4BrO_2$), 220.1 (M−$C_5H_6BrN_3O_2$).

4.2.4. *O*-2-Bromobutyrylfluconazole (1d). The general synthetic method described above afforded **1d** (31.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20 (2H, s), 7.99 (2H, s), 7.10 (1H, d, J = 8.5 Hz), 7.05 (1H, d, J = 2.1 Hz), 6.88 (1H, dd, J = 8.5, 2.1 Hz), 5.39 (2H, d, J = 13.4 Hz), 5.15 (2H, d, J = 13.4 Hz), 4.30 (1H, t, J = 7.5 Hz), 1.49–1.40 (2H, m), 1.15 (3H, t, J = 8.2 Hz). HR-MS (ESI-TOF) calculated for $C_{17}H_{17}BrF_2N_6O_2$ 454.0564, found 455.0531 (M+H, ^{79}Br), 457.0542 (M+H, ^{81}Br). ESI m/z 455.3 (M+H, ^{79}Br), 457.3 (M+H, ^{81}Br), 386.1 (M−$C_2H_2N_3$, ^{79}Br), 388.1 (M−$C_2H_2N_3$, ^{81}Br), 289.3 (M−$C_4H_6BrO_2$), 220.1 (M−$C_8H_8BrN_3O_2$).

4.2.5. *O*-2-Bromovaleroylfluconazole (1e). The general synthetic method described above afforded **1e** (55.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.22 (2H, s), 8.00 (2H, s), 7.15 (1H, d, J = 8.3 Hz), 7.09–6.80 (2H, m), 5.40 (2H, d, J = 16.1 Hz), 5.12 (2H, d, J = 16.1 Hz), 4.38 (1H, t, J = 7.2 Hz), 2.12–2.00 (2H, m), 1.49–1.42 (2H, m), 0.96 (3H, t, J = 7.5 Hz). HR-MS (ESI-TOF) calculated for $C_{18}H_{19}BrF_2N_6O_2$ 468.0721, found 469.0712 (M+H, ^{79}Br), 471.0711 (M+H, ^{81}Br). ESI m/z 469.0 (M+H, ^{79}Br), 471.1 (M+H, ^{81}Br), 400.0 (M−$C_2H_2N_3$, ^{79}Br), 402.1 (M−$C_2H_2N_3$, ^{81}Br), 289.3 (M−$C_5H_8BrO_2$), 220.1 (M−$C_7H_{10}BrN_3O_2$).

4.2.6. *O*-2-Bromohexanoylfluconazole (1f). The general synthetic method described above afforded **1f** (69.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.16 (2H, s), 7.98 (2H, s), 7.09 (1H, d, J = 8.3 Hz), 6.88 (1H, d, J = 2.5 Hz), 6.79 (1H, dd, J = 8.3, 2.5 Hz), 5.38 (2H, d, J = 14.2 Hz), 5.11 (2H, d, J = 14.2 Hz), 4.41 (1H, t, J = 7.7 Hz), 2.05–1.85 (2H, m), 1.33–1.20 (4H, m, methylene envelope), 0.99 (3H, t, J = 7.1 Hz). HR-MS (ESI-TOF) calculated for $C_{19}H_{21}BrF_2N_6O_2$ 482.0877, found 483.0711 (M+H, ^{79}Br), 485.1021 (M+H, ^{81}Br). ESI m/z 483.0 (M+H, ^{79}Br), 485.1 (M+H, ^{81}Br), 414.0 (M−$C_2H_2N_3$, ^{79}Br), 416.1 (M−$C_2H_2N_3$, ^{81}Br), 289.3 (M−$C_6H_{10}BrO_2$), 220.1 (M−$C_8H_{12}BrN_3O_2$).

4.2.7. *O*-2-Bromooctanoylfluconazole (1g). The general synthetic method described above afforded **1g** (75.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20 (2H, s), 7.82 (2H, s), 7.05 (1H, d, J = 8.8 Hz), 6.92 (1H, d, J = 2.7 Hz), 6.80 (1H, dd, J = 8.8, 2.7 Hz), 5.39 (2H, d, J = 15.1 Hz), 5.09 (2H, d, J = 15.1 Hz), 4.31 (1H, t, J = 7.7 Hz), 2.05–1.99 (2H, m), 1.59–1.32 (8H, m, methylene envelope), 0.90 (3H, m). HR-MS (ESI-TOF) calculated for $C_{21}H_{25}BrF_2N_6O_2$ 510.1190, found 511.2011 (M+H, ^{79}Br), 513.3101 (M+H, ^{81}Br). ESI m/z 511.2 (M+H, ^{79}Br), 513.3 (M+H, ^{81}Br), 289.3 (M−$C_{10}H_{16}BrO_2$), 220.1 (M−$C_8H_{12}BrN_3O_2$).

4.2.8. *O*-2-Bromolauroylfluconazole (1h). The general synthetic method described above afforded **1h** (91.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.19 (2H, s), 7.90 (2H, s), 7.08 (1H, d, J = 8.1 Hz), 6.88 (1H, d, J = 2.4 Hz), 6.77 (1H, dd, J = 8.1, 2.4 Hz), 5.32 (2H, d, J = 13.7 Hz), 5.07 (2H, d, J = 13.7 Hz), 4.28 (1H, t, J = 7.5 Hz), 2.13–1.98 (2H, m), 1.59–1.32 (16H, m, methylene envelope), 0.94 (3H, m). HR-MS (ESI-TOF) calculated for $C_{25}H_{33}BrF_2N_6O_2$ 566.1816, found 567.1823 (M+H, ^{79}Br), 569.1821 (M+H, ^{81}Br). ESI m/z 567.3 (M+H, ^{79}Br), 569.4 (M+H, ^{81}Br), 498.3 (M−$C_2H_2N_3$, ^{79}Br), 500.3 (M−$C_2H_2N_3$, ^{81}Br), 289.3 (M−$C_{12}H_{22}BrO_2$), 220.1 (M−$C_{14}H_{24}BrN_3O_2$).

4.2.9. *O*-2-Bromomyristoylfluconazole (1i). The general synthetic method described above afforded **1i** (95.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.59 (2H, s), 7.88 (2H, s), 7.02 (1H, d, J = 8.4 Hz), 6.92 (1H, d, J = 2.3 Hz), 6.81 (1H, dd, J = 8.4, 2.3 Hz), 5.34 (2H, d, J = 14.6 Hz), 5.07 (2H, d, J = 14.6 Hz), 4.31 (1H, t, J = 7.5 Hz), 2.15–1.97 (2H, m), 1.60–1.21 (20H, m, methylene envelope), 0.95 (3H, m). HR-MS (ESI-TOF) calculated for $C_{27}H_{37}BrF_2N_6O_2$ 594.2129, found 595.2121 (M+H, ^{79}Br), 597.2132 (M+H, ^{81}Br). ESI m/z 595.3 (M+H, ^{79}Br), 597.2 (M+H, ^{81}Br), 526.3 (M−$C_2H_2N_3$, ^{79}Br), 528.3 (M−$C_2H_2N_3$, ^{81}Br), 289.4 (M−$C_{14}H_{26}BrO_2$), 220.1 (M−$C_{16}H_{28}BrN_3O_2$).

4.2.10. *O*-11-Bromoundecanoylfluconazole (1j). The general synthetic method described above afforded **1j** (88.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (2H, s), 7.85 (2H, s), 6.95–6.88 (1H, m), 6.87–

6.71 (2H, m), 5.20 (2H, d, $J = 15.5$ Hz), 5.03 (2H, d, $J = 15.5$ Hz), 3.40 (2H, –CH_2Br, t, $J = 7.7$ Hz), 2.32 (2H, COCH_2–, t, $J = 7.7$ Hz), 1.90–1.81 (2H, –CH_2CH$_2$Br, m), 1.61–1.51 (2H, COCH$_2$CH_2–, m), 1.45–1.20 (12H, m, methylene envelope). HR-MS (ESI-TOF) calculated for $C_{24}H_{31}BrF_2N_6O_2$ 552.1660, found 553.1647 (M+H, ^{79}Br), 555.2111 (M+H, ^{81}Br). ESI *m/z* 553.4 (M+H, ^{79}Br), 555.2 (M+H, ^{81}Br), 484.2 (M−$C_2H_2N_3$, ^{79}Br), 486.2 (M−$C_2H_2N_3$, ^{81}Br), 289.3 (M−$C_{11}H_{20}BrO_2$), 220.1 (M−$C_{13}H_{22}BrN_3O_2$).

4.2.11. *O*-2-Chloroethyloxycarbonylfluconazole (1k). The general synthetic method described above afforded **1k** (75.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz) 8.05 (2H, s), 7.95 (2H, s), 7.05–6.90 (2H, m), 6.88–6.80 (1H, m), 5.15 (2H, d, $J = 14.3$ Hz), 5.05 (2H, d, $J = 14.3$ Hz), 4.50 (2H, –OCH_2–, t, $J = 9.2$ Hz), 3.76 (2H, –OCH$_2$CH_2Cl, t, $J = 9.2$ Hz). HR-MS (ESI-TOF) calculated for $C_{16}H_{15}ClF_2N_6O_3$ 412.0862, found 413.0866 (M+H, ^{79}Br), 415.0811 (M+H, ^{81}Br). ESI *m/z* 413.1 (M+H), 344.1 (M−$C_2H_2N_3$), 289.3 (M−$C_3H_4ClO_3$), 220.1 (M−$C_5H_6ClN_3O_3$).

4.2.12. *O*-Salicylfluconazole (1l). The general synthetic method described above afforded **1l** (44.0%) as an amorphous powder; mp 142–143 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 (2H, s), 8.05 (1H, d, $J = 8.3$ Hz), 7.93 (2H, s), 7.56–7.50 (1H, m), 7.31–7.22 (2H, m), 7.07–6.92 (2H, m), 6.87–6.78 (1H, m), 5.21 (2H, d, $J = 15.1$ Hz), 5.08 (2H, d, $J = 15.1$ Hz), 2.15 (3H, s). ESI *m/z* 469.0 (M+H), 426.9 (M−C_2H_3O+H), 399.9 (M−$C_2H_2N_3$), 289.3 (M−$C_9H_7O_4$), 220.1 (M−$C_{11}H_9N_3O_4$).

4.3. General procedure for the synthesis of phosphate triester derivatives of FLC (2a–h)

2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (267 μL, 1.2 mmol) (for **2a**–**e**) or methyl *N*,*N*-diisopropylchlorophosphoramidite (240 μL, 1.2 mmol) (for **2f**–**h**) and diisopropyl ethylamine (209 μL, 1.2 mmol) were added to a solution of alcohol (ROH) (1.0 mmol) in dry DCM (2 mL) under a N_2 atmosphere. After stirring for 20 h, the reaction mixture was partitioned between EA (10 mL) and distilled water (10 mL). The organic layer was then washed with brine (10 mL) and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo and the syrup was dried under vacuum overnight. FLC (200 mg, 0.6 mmol) and 1*H*-tetrazole (84 mg, 1.2 mmol) were dissolved in dry dichloromethane (DCM, 2 mL) under a N_2 atmosphere. The syrup was dissolved in dry DCM (1 mL) and was added to this reaction mixture. After stirring for 3 h, the reaction was cooled to 0 °C and *t*-butyl hydroperoxide (0.3 mL) was added. After stirring for 1 h, the reaction mixture was partitioned between EA (10 mL) and distilled water (10 mL). The organic phase was then washed with brine (10 mL) and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo. The syrup residue was purified by silica gel column chromatography, eluting with hexane and acetone from 3:1 (v/v) to 1:1 (v/v) to yield **2a**–**h** (Scheme 3).

4.3.1. 2-Cyanoethyl-*n*-undecanyl fluconazole phosphate (2a). The general synthetic method described above afforded **2a** (24.0%) as a wax; mp 75.4–77.0 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 (2H, s), 7.93 (2H, s), 7.22–7.15 (1H, m), 6.94–6.83 (1H, m), 6.83–6.72 (1H, m), 5.21 (2H, d, $J = 14.2$ Hz), 5.03 (2H, d, $J = 14.4$ Hz), 4.15 (2H, –OCH_2CH$_2$CN, t, $J = 7.2$ Hz), 3.93 (2H, –OCH_2–, t, $J = 7.7$ Hz), 2.74 (2H, –OCH$_2$CH_2CN, t, $J = 7.2$ Hz), 1.57 (2H, –OCH$_2$-CH_2CH$_2$–, t, $J = 7.6$ Hz), 1.41–1.27 (16H, methylene envelope), 0.92–0.83 (3H, m). HR-MS (ESI-TOF) calculated for $C_{27}H_{38}F_2N_7O_4P$ 593.2691, found 594.2710 (M+H).

4.3.2. 2-Cyanoethyl-ω-undecylenyl fluconazole phosphate (2b). The general synthetic method described above afforded **2b** (36.1%) as a wax; mp 72.6–73.6 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.35 (2H, s), 7.95 (2H, s), 7.20–7.10 (1H, m), 6.91–6.81 (1H, m), 6.81–6.71 (1H, m), 5.88–5.75 (1H, CH$_2$=C*H*–, m), 5.23 (2H, d, $J = 15.1$ Hz), 5.05 (2H, d, $J = 15.2$ Hz), 5.01–4.85 (2H, CH_2=CH–, m), 4.20 (2H, –OCH_2CH$_2$CN, t, $J = 7.5$ Hz), 3.98 (2H, –OCH_2–, t, $J = 7.8$ Hz), 2.72 (2H, –OCH$_2$CH_2CN, t, $J = 7.5$ Hz), 2.11–2.00 (2H, CH$_2$=CHCH_2–, m), 1.58 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 7.2$ Hz), 1.40–1.15 (12H, methylene envelope). HR-MS (ESI-TOF) calculated for $C_{27}H_{36}F_2N_7O_4P$ 591.2534, found 592.2541.

4.3.3. 11-Bromoundecanyl-2-cyanoethyl fluconazole phosphate (2c). The general synthetic method described above afforded **2c** (28.1%) as a wax; mp 77.2–78.9 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.32 (2H, s), 7.88 (2H, s), 7.20–7.10 (1H, m), 6.92–6.82 (1H, m), 6.82–6.71 (1H, m), 5.22 (2H, d, $J = 16.0$ Hz), 5.05 (2H, d, $J = 16.0$ Hz), 4.15 (2H, –OCH_2CH$_2$CN, t, $J = 8.1$ Hz), 3.98 (2H, –OCH_2–, t, $J = 6.4$ Hz), 3.43 (2H, –CH_2Br, t, $J = 8.0$ Hz), 2.71 (2H, –OCH$_2$CH_2CN, t, $J = 8.1$ Hz), 1.88–1.80 (2H, –CH_2CH$_2$Br, m), 1.75–1.63 (2H, COCH$_2$CH_2–, m), 1.43–1.21 (14H, m, methylene envelope). HR-MS (ESI-TOF) calculated for $C_{27}H_{37}BrF_2N_7O_4P$ 671.1796, found 672.1855 (M+H, ^{79}Br), 674.1819 (M+H, ^{79}Br).

4.3.4. 2-Cyanoethyl-tetradecanyl fluconazole phosphate (2d). The general synthetic method described above afforded **2d** (27.3%) as a wax; mp 79.2–83.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.33 (2H, s), 7.88 (2H, s), 7.20–7.11 (1H, m), 6.90–6.80 (1H, m), 6.80–6.71 (1H, m), 5.22 (2H, d, $J = 14.9$ Hz), 5.10 (2H, d, $J = 14.8$ Hz), 4.18 (2H, –OCH_2CH$_2$CN, t, $J = 7.8$ Hz), 3.96 (2H, –OCH_2–, t, $J = 7.4$ Hz), 2.78 (2H, –OCH$_2$CH_2-CN, t, $J = 7.7$ Hz), 1.56 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 7.4$ Hz), 1.38–1.18 (22H, methylene envelope), 0.86 (3H, t, $J = 7.5$ Hz). HR-MS (ESI-TOF) calculated for $C_{30}H_{44}F_2N_7O_4P$ 635.3160, found 636.3221 (M+H). ESI *m/z* 636.4 (M+H).

4.3.5. 2-Cyanoethyl-*n*-octyl fluconazole phosphate (2e). The general synthetic method described above afforded **2e** (3.9%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz) 8.35 (2H, s), 7.91 (2H, s), 7.12–7.22 (1H, m), 6.95–6.85 (1H, m), 6.85–6.75 (1H, m), 5.23 (2H, d, $J = 15.1$ Hz),

5.07 (2H, d, $J = 15.1$ Hz), 4.18 (2H, –OCH_2CH$_2$CN, t, $J = 6.8$ Hz), 3.95 (2H, –OCH_2–, t, $J = 8.3$ Hz), 2.70 (2H, –OCH$_2$CH_2CN, t, $J = 6.8$ Hz), 1.61 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 8.3$ Hz), 1.38–1.25 (10H, methylene envelope), 0.86 (3H, t, $J = 7.7$ Hz). HR-MS (ESI-TOF) calculated for $C_{24}H_{32}F_2N_7O_4P$ 551.2211, found 552.2219 (M+H).

4.3.6. Methyl-undecanyl fluconazole phosphate (2f). The general synthetic method described above afforded **2f** (28.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.33 (2H, s), 7.95 (2H, s), 7.21–7.14 (1H, m), 6.93–6.82 (1H, m), 6.82–6.73 (1H, m), 5.25 (2H, d, $J = 14.7$ Hz), 5.05 (2H, d, $J = 14.7$ Hz), 3.94 (2H, –OCH_2–, t, $J = 7.8$ Hz), 3.41 (3H, –OCH$_3$, s), 1.59 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 7.6$ Hz), 1.45–1.22 (16H, methylene envelope), 0.89 (3H, t, $J = 7.2$ Hz). ESI *m/z* 555.2 (M+H).

4.3.7. Methyl-ω-undecylenyl fluconazole phosphate (2g). The general synthetic method described above afforded **2g** (36.7%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 (2H, s), 7.92 (2H, s), 7.21–7.11 (1H, m), 6.93–6.83 (1H, m), 6.82–6.72 (1H, m), 5.87–5.71 (1H, CH$_2$=CH–, m), 5.25 (2H, d, $J = 15.4$ Hz), 5.05 (2H, d, $J = 15.5$ Hz), 5.06–4.89 (2H, CH_2=CH–, m), 3.96 (2H, –OCH_2–, t, $J = 7.8$ Hz), 3.40 (3H, –OCH$_3$, s), 2.15–2.03 (2H, CH$_2$=CHCH_2–, m), 1.58 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 7.2$ Hz), 1.43–1.17 (12H, methylene envelope). ESI *m/z* 553.4 (M+H).

4.3.8. Methyl-*n*-octyl fluconazole phosphate (2h). The general synthetic method described above afforded **2h** (38%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 (2H, s), 7.99 (2H, s), 7.25–7.16 (1H, m), 7.01–6.91 (1H, m), 6.87–6.71 (1H, m), 5.25 (2H, d, $J = 14.7$ Hz), 5.11 (2H, d, $J = 14.7$ Hz), 3.99 (2H, –OCH_2–, t, $J = 7.8$ Hz), 3.57 (3H, OCH$_3$, s), 1.69 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 8.1$ Hz), 1.43–1.29 (10H, methylene envelope), 0.91 (3H, t, $J = 8.3$ Hz). MS (ESI) calculated for $C_{22}H_{31}F_2N_6O_4P$ 512.2, found 513.2 (M+H).

4.4. General procedure for the synthesis of phosphate diester derivatives of FLC (3a–d)

Ammonium hydroxide (57 μL, 0.48 mmol) was added to a solution of compound **2** (114.8 mg, 0.19 mmol) in methanol (1 mL) and DCM (1 mL). After stirring for 24 h, the solvent was evaporated in vacuo. The syrup residue was centrifuged and lyophilized to afford **3** (Scheme 2).

4.4.1. *n*-Undecanyl fluconazole phosphate (3a). The general synthetic method described above afforded **3a** (65.6%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.28 (2H, s), 7.91 (2H, s), 7.21–7.13 (1H, m), 6.92–6.82 (1H, m), 6.82–6.71 (1H, m), 5.20 (2H, d, $J = 15.3$ Hz), 5.01 (2H, d, $J = 15.4$ Hz), 3.95 (2H, –OCH_2–, t, $J = 8.1$ Hz), 1.59 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 8.1$ Hz), 1.41–1.27 (16H, methylene envelope), 0.95–0.86 (3H, m). HR-MS (ESI-TOF) calculated for $C_{24}H_{35}F_2N_6O_4P$ 540.2425, found 541.2431 (M+H).

4.4.2. ω-Undecylenyl fluconazole phosphate (3b). The general synthetic method described above afforded **3b** (65.2%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.28 (2H, s), 7.88 (2H, s), 7.15–7.00 (2H, m), 6.89–6.75 (1H, m), 5.89–5.76 (1H, CH$_2$=CH–, m), 5.25 (2H, d, $J = 14.2$ Hz), 5.03 (2H, d, $J = 14.2$ Hz), 5.00–4.86 (2H, CH_2=CH–, m), 3.99 (2H, –OCH_2–, t, $J = 8.1$ Hz), 2.10–1.99 (2H, CH$_2$=CHCH_2–, m), 1.60 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 7.5$ Hz), 1.44–1.18 (12H, methylene envelope). HR-MS (ESI-TOF) calculated for $C_{24}H_{33}F_2N_6O_4P$ 538.2269, found 539.2270 (M+H).

4.4.3. 11-Bromoundecanyl fluconazole phosphate (3c). The general synthetic method described above afforded **3c** (100.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.26 (2H, s), 7.89 (2H, s), 7.12–7.00 (1H, m), 6.93–6.84 (1H, m), 6.83–6.72 (1H, m), 5.20 (2H, d, $J = 14.9$ Hz), 5.02 (2H, d, $J = 14.9$ Hz), 3.91 (2H, –OCH_2–, t, $J = 8.4$ Hz), 3.47 (2H, –CH_2Br, t, $J = 7.8$ Hz), 1.87–1.81 (2H, –CH_2CH$_2$Br, m), 1.76–1.65 (2H, COCH$_2$CH_2–, m), 1.45–1.23 (14H, m, methylene envelope). ESI *m/z* 619.2 (M+H, ^{79}Br), 621.2 (M+H, ^{81}Br).

4.4.4. *n*-Tetradecanyl fluconazole phosphate (3d). The general synthetic method described above afforded **3d** (100.0%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz) 8.25 (2H, s), 7.86 (2H, s), 7.18–7.09 (1H, m), 6.92–6.83 (1H, m), 6.82–6.73 (1H, m), 5.20 (2H, d, $J = 15.1$ Hz), 4.99 (2H, d, $J = 14.9$ Hz), 3.97 (2H, –OCH_2–, t, $J = 7.7$ Hz), 1.57 (2H, –OCH$_2$CH_2CH$_2$–, t, $J = 7.7$ Hz), 1.41–1.98 (22H, methylene envelope), 0.88 (3H, t, $J = 7.9$ Hz). ESI *m/z* 583.3 $(M+H)^+$.

4.5. General procedure for the synthesis of carbohydrate phosphate triester derivatives of FLC (4a and 4b)

Benzylamine (BnNH$_2$) (163 μL, 1.5 mmol) was added to a solution of β-D-glucosepentaacetate **(7a)** or 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **(7b)** (1 mmol) in THF (3 mL). After stirring overnight, the reaction mixture was diluted with cold, distilled water and extracted with DCM twice (2 × 5 mL). The combined organic phase was, respectively, washed with ice-cold diluted hydrochloric acid, saturated sodium bicarbonate (5 mL), brine (5 mL), and distilled water (5 mL). The combined extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. The syrup was dried in a vacuum overnight to afford **8a** and **8b** (Scheme 3). 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (236.7 μL, 1 mmol) and diisopropyl ethylamine (174.3 μL, 1 mmol) were added to a solution of **8a** or **8b** (0.5 mmol) in dry DCM (2 mL). After stirring for 20 h, the reaction mixture was extracted with EA (10 mL) and distilled water (10 mL). The organic phase was then washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the syrup was dried under vacuum overnight to yield **9a** and **9b** (Scheme 3). FLC (100 mg, 0.3 mmol) and 1*H*-tetrazole (42 mg, 0.6 mmol) were dissolved in dry DCM (1 mL) under a N_2 atmosphere. The syrup (**9a** or **9b**) was dissolved in dry DCM (0.5 mL) and added to the reaction

mixture. After stirring for 3 h, the reaction mixture was cooled to 0 °C and *t*-butyl hydroperoxide (0.15 mL) was added. After stirring for 1 h, the reaction mixture was partitioned between EA (10 mL) and distilled water (10 mL). The organic layer was then washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed in vacuo. The syrup residue was purified using silica gel column chromatography, eluting with hexane/acetone from 2:1 (v/v) to 1:1 (v/v) to afford **4a** or **4b** (Scheme 3).

4.5.1. 2-Cyanoethyl-[1-(β-D-2,3,4,6-glucosyltetraacetate)]-fluconazole phosphate (4a). The general synthetic method described above afforded **4a** (13.9%) as a syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73 (2H, s), 7.56 (2H, s), 7.55–7.21 (3H, m), 5.95–5.82 (1H, anomeric proton, m), 5.60–5.42 (2H, m), 5.10 (2H, d, J = 15.1 Hz), 4.90 (2H, d, J = 15.1 Hz), 4.41–4.08 (6H, m), 2.78 (2H, –OCH_2CH_2CN, t, J = 7.8 Hz), 2.15–2.10 (12H, overlap). HR-MS (ESI-TOF) calculated for $C_{30}H_{34}F_2N_7O_{13}P$ 769.1920, found 770.1927 (M+H).

4.5.2. 2-Cyanoethyl-[1-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosidyl)]fluconazole phosphate (4b). The general synthetic method described above afforded **3b** (8.3%) as a wax; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.81 (2H, s), 7.59 (2H, s), 7.52–7.19 (3H, m), 5.91–5.80 (1H, anomeric proton, m), 5.59–5.43 (2H, m), 5.05 (2H, d, J = 14.7 Hz), 4.92 (2H, d, J = 14.7 Hz), 4.42–4.33 (2H, m), 4.39–4.05 (4H, m), 2.75 (2H, –OCH_2CH_2CN, t, J = 7.3 Hz), 2.12–2.06 (12H, overlap). HR-MS (ESI-TOF) calculated for $C_{30}H_{35}F_2N_8O_{12}P$ 768.2080, found 769.2068 (M+H).

4.5.3. Physicochemical properties. Physicochemical properties were estimated using the KowWin program (Version 1.67) and estimation methodology developed at Syracuse Research Corporation (Environmental Chemistry Center, KOWWIN—The Octanol–Water Partition Coefficient Program, PC-Computer software, Running Ridge Road, North Syracuse, NY, 1999). The Log K_{ow} (KowWin) program estimates the log octanol/water partition coefficient (log P) of organic chemicals using an atom/fragment contribution method.[48] KowWin uses a 'fragment constant' methodology to predict log P. In a 'fragment constant' method, a structure is divided into fragments (atom or larger functional groups) and coefficient values of each fragment or group are summed together to yield the log P estimate. To confirm the validity of the log P calculation, the reported experimental data were compared with acquired data for some of the compounds. The experimental data were in agreement with the calculated log P values.

The Dermal Permeability Coefficient Program (DermWin Version 1.43) was used to estimate the dermal permeability coefficient (K_p) and the dermally absorbed dose per event (DAevent) of organic compounds. DermWin estimates a Log K_{ow} for every SMILES notation by using the estimation engine from the KowWin Program based on the general equation, $\text{Log}K_p = -2.72 + 0.71\,\text{Log}K_{ow} - 0.0061$ MW, where Log K_{ow} and MW are estimated partition coefficient and molecular weight for each compound.

The HYDROWIN (Version 1.67) (Mill, T., Haag, W., Penwell, P., Pettit, T., Johnson, H. EPZ Contract no 68-02-4254. Menlo Park, CA: SRI International, 1987) was used to estimate the half-lives based on the total acid-catalyzed rate constant and the prediction methodology developed for the US Environment Protection Agency. The half-lives for acid-catalyzed rate constants were calculated at pH 5.6 from the general equation, Half-life = $0.6931/(K_b)(1.0\text{E}{-}8.4)$, where 1.0E−8.4 is the OH^- concentration in water at pH 5.6.

4.6. In vitro antifungal activity

4.6.1. Microorganisms. *C. albicans* ATCC 14053, *A. niger* ATCC 16404, and *C. neoformans* ATCC 66031 were obtained from American Type Culture Collection, Manassas, Virginia. Stock cultures were kept on Sabouraud dextrose agar (SDA; Becton-Dickinson and Co., Sparks, Maryland). Subcultures were prepared on SDA at 35–37 °C. Suspension cultures were prepared by inoculation of single colonies in 7 mL of normal saline solution. Prior to preparation of susceptibility assays, yeast cells were resuspended in normal saline to make a transmittance of 73–75% at 530 nm that provide equivalent concentration of 10^6 cells/mL and spores of *A. niger* in saline medium to produce a similar transmittance at 530 nm compared to the control tube. The media were RPMI (RPMI 1640; ICN Biomedical, Aurora, Ohio) adjusted to pH 6.9 and Sabouraud dextrose broth (SDB; Becton Dickinson and Co., Sparks, Maryland).

4.6.2. Chemicals and antifungal agents. AMB was purchased from Acros, New Jersey, USA, and was kept as a 5 μM stock in DMSO at 0 °C and used during one week of preparation. FLC was purchased from Medisa Inc., New York, USA, or was provided from Vera Laboratories Ltd, Hyderabad, India, and was kept as a 20 μM stock solution at 0 °C. Test compounds were dissolved in DMSO (0.56 mg/mL) and stored at 0 °C. Working dilutions were made in RPMI or SDB medium. Higher concentrations of compound were used for compounds with weak antifungal activities. The final maximum concentration of DMSO in the assays was 5% (v/v). DMSO was not inhibitory to the organisms in the concentrations tested.

4.6.3. Susceptibility testing. Microdilutions for control experiments with *C. albicans*, *A. niger,* and *C. neoformans* were the modified method of National Committee for Clinical Laboratory Standards (NCCLS) method as described by Galgiani[49] and by the more recent NCCLS M27-A microdilution methods.[50] as described previously.[51] Dilutions were prepared in 0.1 mL of RPMI or SDB; the inocula were either 10^4 *C. albicans* or *C. neoformans* cells or *A. niger* spores. The tubes were incubated for 24–48 h at 36 ± 1 °C, and turbidity was read visually. MICs were calculated in comparison to growth control as the lowest concentration that shows inhibition for AMB, FLC, and the test compounds.

Acknowledgements

We are grateful to the Slater Center for Biomedical Technology for a grant to Keykavous Parang for carrying out this research at University of Rhode Island. We thank Vera Laboratories Ltd, Hyderabad, India, for providing us some of FLC required for this study.

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