Silicon Incorporated Morpholine Antifungals: Design, Synthesis, and Biological Evaluation

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S Supporting Information

ABSTRACT: Known morpholine class antifungals (fenpropimorph, fenpropidin, and amorolfine) were synthetically modified through silicon incorporation to have 15 sila-analogues. Twelve sila-analogues exhibited potent antifungal activity against different human fungal pathogens such as *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Cryptococcus neoformans*, and *Aspergillus niger*. Sila-analogue 24 (fenpropimorph analogue) was the best in our hands, which showed superior fungicidal potential than fenpropidin, fenpropimorph, and amorolfine. The mode of action of sila-analogues was similar to morpholines, i.e., inhibition of sterol reductase and sterol isomerase enzymes of ergosterol synthesis pathway.

Sila-analogues of morpholine antifungals

KEYWORDS: Antifungal drugs, *Candida albicans*, ergosterol biosynthesis, morpholines, sila-analogues

Steep rise in invasive fungal infections (IFI) mainly in immunocompromised patients, change in epidemiology of IFI, emergence of fungal pathogens, which were previously insignificant, development of resistance against currently used antifungal drugs, and certain drawbacks (like less clinical efficacy, acute and chronic side effects) associated with them necessitate the development of new antifungal drugs.^{1,2} Similarly, in agriculture, fungal infections is a major cause for crop loss with consequential heavy economic burden worldwide. Though several groups of fungicides are available, development of resistance against most of them stresses on the need to explore new antifungal agents.³

An ideal antifungal agent is one that selectively interacts with a fungal target not found in other eukaryotic cells. The fungal cell wall and cell membrane are attractive therapeutic targets as cell wall constituents such as chitin and major membrane lipid ergosterol are not present in mammalian cells. For instance, from currently used antifungals, three classes of compounds inhibit ergosterol synthesis through selective inhibition of different enzymes involved in the biosynthesis pathway. Allylamines such as terbinafine and naftifine act on squalene epoxidase, and azoles inhibit cytochrome P450 enzyme lanosterol 14- α -demethylase, whereas morpholines act on two different enzymes of this pathway, viz., sterol Δ^{14} reductase and sterol Δ^7 - Δ^8 isomerase (Figure 1).⁴ Thus, morpholines seem to be ideal antifungals, as acquiring resistance against them will be difficult for a pathogen because of the requirement to mutate two genes of these enzymes. However, only one drug, amorolfine, from this class is

used clinically, and its use is restricted to topical application for nail infections, the reason is that though quite effective *in vitro* against different human pathogens, it is ineffective against IFI due to rapid metabolism inside the host.⁵ Nevertheless, in agriculture, aldimorph, fenpropimorph, fenpropidin, tridemorph, and dodemorph from this class are in use as fungicides (Figure 1). With this background, we synthesized modified morpholines by incorporating silicon to increase their efficacy and stability.

The introduction of bioisostere is a key strategy used by medicinal chemists during lead optimization process in order to improve the desired biological and physical properties of a potential compound without altering its structure significantly,^{6,7} which may lead to improvement in bioactivity and stability or reduction in toxicity of the compound. Chemists have used silicon as a classical bioisostere for carbon as it has similar steric and electronic features and has the same valency as carbon.^{8–12} The change in biological activities due to sila-substitution may be attributed to larger bond length, altered bond angles, and different ring conformations due to larger covalent radius of silicon over carbon and increased lipophilicity, which in turn will increase its tissue distribution, particularly uptake through membranes.^{13–15} In our group, we are interested in making silicon analogues of biologically active compounds so as to improve their drug-like properties.^{16–18} Acker et al. have

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Figure 1. Ergosterol synthesis pathway and known inhibitors of the pathway enzymes.

previously reported the synthesis of siliconized antifungals in the patent literature, but their use was restricted to fungal plant pathogens.¹⁹ Specifically, we have reasoned our design around fenpropimorph and amorolfine, which are closely related antifungals. Generally metabolism of fenpropidin takes place through hydroxylation of the piperidine ring or hydroxylation and oxidation of the methyls of aromatic *t*-butyl group (Figure 2).²⁰ Closely related amorolfine also undergoes oxidation at isopentyl group present at the *p*-position of the aromatic ring.²¹

We hypothesized that incorporation of silicon at these metabolic soft spots in morpholine antifungals will help to overcome their drawbacks and give more potent and stable

compounds (Figure 2). In general, all the silicon analogues are expected to be more lipophilic compared with fenpropidin, fenpropimorph, and amorolfine. Based on calculated cLogP values, incorporation of one silicon atom showed only slight increase in lipophilicity, but two silicon atoms incorporation resulted in large increase in lipophilicity with respect to fenpropidin (see Table S2 in the Supporting Information). Incorporation of silicon in to heterocycle (piperidine ring) has more effect when compared to silicon incorporation in the aromatic ring. Sometimes, high lipophilicity can be detrimental to the druggable properties such as solubility. This undesired increase in lipophilicity can be counterbalanced by the addition of polar groups to the molecule, for example, morpholine containing silicon analogue showed lower cLogP values with respect to all three fungicides fenpropidin, amorolfine, and fenpropimorph.

To begin with, 4-(trimethylsilyl) benzaldehyde **1** was first prepared from 1,4-dibromobenzene as reported earlier.²² Treatment of **1** with ylide ethyl-2-(triphenyl phosphanylidene) propanoate in anhydrous toluene at 110 °C, afforded ethyl 2-methyl-3-(4-(trimethylsilyl)phenyl) acrylate, **2**. Hydrogenation of **2** in the presence of 10% Pd/C in EtOH followed by ester hydrolysis using LiOH in THF-MeOH-H₂O led to formation of carboxylic acid **3**. Carboxylic acid **3** was then coupled with piperidine under standard conditions using EDC·HCl and HOBT in CH₂Cl₂ to give the amide **4**. Reduction of amide **4** using LAH in THF afforded the sila-amine **5** (Scheme 1). The furnished carboxylic acid **3** was further exploited to synthesize a library of sila-amines **15–24** (Scheme 1, see details in the Supporting Information). The sulfone derivative **17** was prepared from **16** by *m*-CPBA oxidation. In a similar way, carboxylic acid **6**^{23,24} and **7**^{23,24} on coupling with 4,4-dimethyl-4-silapiperidine **8**^{17,18} using standard protocol followed by reduction of the resulting amide gave amines **11** and **12**, respectively. Carboxylic acid **6** was

Figure 2. Metabolism of fenpropidin and rationale for our design.

Scheme 1. Synthesis of Silicon Incorporated Morpholines

coupled to (dimethyl(phenyl)silyl)methanamine **13** to give the amide **14**. However, reduction of amide carbonyl of **14** to give corresponding amine was not successful. The structures of the compounds were confirmed by ^1H NMR, ^{13}C NMR, and HR-MS spectra.

All the synthesized sila-analogues were tested for antifungal activity against different human pathogenic yeasts and filamentous fungi by Clinical Laboratory Standards Institute's (CLSI) broth microdilution assay (CLSI document M27-A3 and CLSI M38-A2).^{25,26} The results in terms of IC_{50} , i.e., concentration causing 50% growth inhibition, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) are given in Table 1. From the 15 sila-compounds synthesized, 12 compounds, namely, **5**, **11**, **12**, **15**, **16**, and **18–24**, exhibited potent antifungal activity against *Candida albicans* ATCC 24433, *C. albicans* ATCC 10231,

Candida glabrata NCYC 388, *Candida tropicalis* ATCC 750, *Cryptococcus neoformans* ATCC 34664, and *Aspergillus niger* ATCC 10578 (Table 1). No antifungal effect was observed ($\text{MIC} \geq 256 \mu\text{g/mL}$) for three compounds, viz., **9**, **14**, and **17** against all the pathogens tested. Compounds **9** and **14** are amides suggesting that the basic tertiary nitrogen is essential for the activity. In the case of compound **17**, presence of polar sulfone moiety on heterocycle was found to be detrimental for the activity. The sila fenpropimorph analogue **24** was most effective followed by sila fenpropidin analogues **5** and **15**. It is worth highlighting that silicon incorporation on aromatic ring seems to be more effective compared to silicon incorporation in heterocycle. Compound **24** exhibited better antifungal activity than fluconazole and morpholine fungicides fenpropimorph and fenpropidin against all the pathogens. Further, the MIC values of

Table 1. Antifungal Activity of the Sila-Morpholine Analogues against Different Human Pathogenic Fungi

compd	<i>Candida albicans</i> ATCC 24433			<i>Candida albicans</i> ATCC 10231			<i>Cryptococcus neoformans</i> ATCC 34664			<i>Candida glabrata</i> NCYC 388			<i>Candida tropicalis</i> ATCC 750			<i>Aspergillus niger</i> ATCC 10578		
	IC ₅₀ ^a	MIC ^b	MFC ^c	IC ₅₀	MIC	MFC	IC ₅₀	MIC	MFC	IC ₅₀	MIC	MFC	IC ₅₀	MIC	MFC	IC ₅₀	MIC	MFC
5	0.12	0.25	2	0.11	0.25	32	0.0625	0.125	8	0.035	0.125	8	1	4	64	41.24	64	128
11	1.85	4	8	0.31	0.5	0.5	0.875	2	4	1.52	4	8	16	64	64	13.82	32	64
12	1.68	4	16	0.28	0.5	64	1.4	2	4	1.47	4	4	13.8	64	256	0.16	0.5	64
15	0.74	1	8	0.26	0.5	8	0.125	0.5	2	0.144	0.5	16	0.5	8	16	105.93	128	>256
16	0.245	1	8	0.5	1	2	0.80	2	64	0.25	0.5	8	0.25	2	>256	1	2	>256
18	1.25	4	8	0.45	1	4	1.98	8	64	1.75	8	16	44.8	64	>256	0.5	2	>256
19	0.396	1	2	0.19	0.25	4	0.93	2	16	0.196	0.5	8	0.198	4	256	0.25	0.5	256
20	1.55	4	8	1	2	2	3.77	8	8	1.63	2	4	5.87	16	16	1.25	2	64
21	5.13	8	8	1.59	2	2	6.9	16	16	5.92	8	16	1.50	64	256	4.5	8	>256
22	3.75	8	32	0.57	2	4	1.5	4	32	1.85	4	>256	4.85	16	256	64	256	>256
23	0.97	2	8	0.31	0.5	2	0.25	1	4	0.319	1	32	2.76	16	128	50.08	64	256
24	0.043	0.125	0.5	0.25	0.5	1	0.079	0.125	2	0.017	0.125	2	0.164	0.5	64	0.5	2	256
9, 14, 17	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256	≥256
fenpropidin	0.19	0.25	0.5	0.27	1	2	0.22	1	32	0.1	0.5	16	0.125	4	256	16	32	128
fenpropimorph	0.21	0.5	4	0.833	2	2	0.25	1	64	0.25	1	128	0.125	2	>256	1	4	>256
amorolfine	0.028	1	8	0.1	0.25	32	0.031	0.125	64	0.038	0.125	32	0.173	0.5	>256	0.125	0.5	>256
fluconazole	0.73	2	4	4.21	8	16	4.6	32	>256	16	128	>256	64	>256	>256	>256	>256	>256

^aIC₅₀ concentration causing 50% growth inhibition. All the values are in $\mu\text{g/mL}$. The assay was done twice in triplicate. ^bMIC, minimum inhibitory concentration. All the values are in $\mu\text{g/mL}$. The assay was done twice in triplicate. ^cMFC, minimum fungicidal concentration. All the values are in $\mu\text{g/mL}$. The assay was done twice in triplicate.

Table 2. Effect of 24 and Amorolfine Treatment on the Sterol Profile of *Candida albicans* ATCC 24433^a

	control	compound 24 0.125 $\mu\text{g/mL}$	compound 24 0.25 $\mu\text{g/mL}$	amorolfine 0.125 $\mu\text{g/mL}$	amorolfine 0.25 $\mu\text{g/mL}$
squalene	07.17 $\pm$ 0.35	21.52 $\pm$ 1.37	13.82 $\pm$ 0.81	22.04 $\pm$ 1.07	17.08 $\pm$ 1.31
ergosterol	73.34 $\pm$ 2.53	15.52 $\pm$ 0.8	11.12 $\pm$ 0.54	30.16 $\pm$ 1.48	06.13 $\pm$ 0.29
cholesta-8,24-dien-3-ol, 4-methyl-(3a',4a')-,	07.19 $\pm$ 0.65	05.36 $\pm$ 0.61	27.78 $\pm$ 1.2	12.26 $\pm$ 0.73	12.03 $\pm$ 0.67
ergosta-5,8-dien-3-ol, (3a')	05.15 $\pm$ 0.44	ND ^b	ND	ND	ND
cholesta-5,24-dien-3-ol	03.58 $\pm$ 0.52	15.34 $\pm$ 0.86	0.87 $\pm$ 0.18	0.44 $\pm$ 0.1	ND
ergosta-8,14-dien-3-ol (ignosterol)	ND	07.23 $\pm$ 0.68	42.29 $\pm$ 2.35	ND	11.11 $\pm$ 0.78
ergosta-5,8,22-trien-3-ol,(3a',22E)- (Lichesterol)	ND	29.84 $\pm$ 1.33	ND	08.26 $\pm$ 0.33	32.13 $\pm$ 1.95
ergostenol	ND	05.19 $\pm$ 0.33	04.12 $\pm$ 0.38	11.56 $\pm$ 0.41	05.71 $\pm$ 0.42
ergosta-7,22-dien-3-ol,(3a',22E)-	ND	ND	ND	15.28 $\pm$ 0.3	15.81 $\pm$ 1.1
unidentified/other sterols	03.57 $\pm$ 0.57	ND	ND	ND	ND

^aAll the values mentioned here are average % determined from two experiments with standard error of the mean. ^bND, Not detected.

24 were comparable or better than amorolfine with more potent fungicidal effect (MFC) against all the tested strains.

Morpholine class of antifungals act on two enzymes of ergosterol biosynthesis pathway, viz., sterol Δ^{14} reductase and sterol Δ^7 - Δ^8 isomerase, leading to ergosterol depletion and accumulation of intermediates ignosterol (Figure 1) and lichesterol.^{27,28} To check whether the present sila-analogues act in a similar way, *C. albicans* ATCC 24433 cells were grown in the presence of different subinhibitory (<MIC) concentrations of 5, 19, 24, and amorolfine and the cellular sterols were extracted and measured spectrophotometrically.²⁹ Ergosterol and the late sterol intermediate 24(28) dehydroergosterol (24(28)DHE) in a sample shows characteristic four-peaked curve in a spectrometric scan between wavelengths 230–300 nm.²⁹ In the presence of compounds 5, 19, and 24, a dose-dependent decrease in the height of the absorbance peaks was observed indicating decrease in the ergosterol content in *Candida* cells (Figure S1 in Supporting Information). Accumulation of specific intermediates was checked by GC–MS quantification of cellular sterols in samples of 24 and amorolfine treatment. Results confirmed decrease in ergosterol content and also showed concomitant increase in the concentration of ignosterol and/or lichesterol (Table 2). The accumulation of these intermediates was like amorolfine treated cells implying the same mode of action, i.e., inhibition of sterol reductase and sterol isomerase. More accumulation of lichesterol (32.13%) than ignosterol (11.11%) for amorolfine indicated stronger action on isomerase enzyme than reductase (Table 2), whereas, in the case of 24, accumulation of ignosterol (42.29%) can be attributed to the major effect on sterol reductase at higher concentration. Depletion of ergosterol compromises membrane integrity, affects membrane proteins functions due to membrane instability, and leads to cessation of growth.

The % microsomal stability of selected compounds was evaluated after 30 min incubation with mouse as well as human liver microsomes. All the tested compounds did not show good stability in mouse liver microsomes. However, three compounds with silicon incorporation (15, 18, and 24) showed relatively better stability than fenpropidin (Table 3). In case of human liver microsomes, metabolic stability was improved including for fenpropidin. Compound 18 showed better stability in human liver microsomes than fenpropidin, whereas compound 24 was found to be slightly inferior to fenpropidin. Thus, incorporation of silicon in the piperidine ring seems to be beneficial toward improving the metabolic stability.

Table 3. % Metabolic Stability of the Compounds in Liver Microsomes (after 30 min)

compds	metabolic stability	
	mouse	human
15	5.1	26.0
18	17.3	44.5
24	6.2	27.3
fenpropidin	1.1	34.4

To determine that the compounds are selectively toxic to the fungal cells and not to mammalian cells, hemolysis assay³⁰ using RBCs and MTT assay³⁰ using HEK293 cells were carried out. RBC hemolysis was not observed for any of the tested compounds up to 128 $\mu\text{g/mL}$, whereas partial hemolysis was observed for 19, 20, and 21 at 256 $\mu\text{g/mL}$ (Supporting Information Table S1). As this concentration was many folds higher than the MICs of the compounds against different pathogens, they may be considered safe. In the case of lead compound 24, only negligible hemolysis ($\sim 7\%$) was observed at 512 $\mu\text{g/mL}$. The concentrations of the compounds required for 50% reduction in growth of HEK293 cells were also far higher than the MICs against fungi. Among the tested compounds, only compound 20 was found to be toxic to some extent against HEK293 cells with IC_{50} of 9.81 $\mu\text{g/mL}$ (Supporting Information Table S1).

In conclusion, total 15 silicon analogues of morpholine antifungals were designed and synthesized. Twelve compounds, viz., 5, 11, 12, 15, 16, and 18–24, exhibited potent antifungal activity against different fungal pathogens. The compounds act similar to morpholines by targeting sterol reductase and sterol isomerase of ergosterol synthesis pathway and cause depletion of ergosterol. Compound 24 showed better antifungal activity than fenpropidin, fenpropimorph, and amorolfine against different yeast and filamentous human pathogens and was identified as the lead. Further optimization of this series is in progress.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00245.

Spectrophotometric sterol analysis, details of biological assays including cytotoxicity and metabolic stability, and experimental procedures and characterization data for all the new compounds (PDF)

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Notes

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■ ABBREVIATIONS

RBCs, red blood cells; HEK293, Human Embryonic Kidney 293 cells

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