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Preliminary communication

N-Alkylated 2,3,3-trimethylindolenines and 2-methylbenzothiazoles. Potential lead compounds in the fight against *Saccharomyces cerevisiae* infections

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ABSTRACT

The synthesis of a variety of *N*-alkylated 2,3,3-trimethylindolenines and 2-methylbenzothiazoles is reported herein. Their potential as antifungal agents is evaluated by preliminary screening against *Saccharomyces cerevisiae* (*S. cerevisiae*), *Schizosaccharomyces pombe* (*S. pombe*), and *Candida albicans* (*C. albicans*). Statistical analyses illustrate a strong relationship between chain length and growth inhibition for *S. cerevisiae* and *S. pombe* ($p < 0.0001$ in every case).

Of particular interest is the activity of both sets of compounds against *S. cerevisiae*, as this is emerging as an opportunistic pathogen, especially in immunosuppressed and immunocompromised patients. Bioassays were set up to compare the efficacy of our range of *N*-alkylated compounds against classic antifungal agents; Amphotericin B and Thiabendazole.

1. Introduction

The rise in number of immunocompromised patients (either through infections, nutritional irregularities or medical treatment) has led to an increase in opportunistic fungal infections [1]. This in turn, is forcing health care professionals to seek more aggressive forms of chemotherapeutic treatments, which inevitably leads to fungal infections with greater resistance to current therapies. Although systemic antifungal agents have been available since the 1950s, the end of the last century saw the development of a new generation of antifungal agent; the triazoles; which revolutionised clinical mycology. Itraconazole **1** and Fluconazole **2** (Fig. 1) were two such triazoles, licensed by the Food and Drug Administration (FDA) for topical use [2]. The dawn of the new millennium brought the new echinocandin antifungals, such as Micafungin **3**, as shown in Fig. 2. These drugs inhibit cell wall synthesis, in particular the synthesis of (1,3)-β-D-glucan; their structure being based on natural lipopeptide products [3]. The shift towards natural products is not particularly surprising; these appear to be more acceptable to both the pharmaceutical industry and patients from a toxicity viewpoint. Indeed many natural products which are being derived from plants, are being identified as antifungal agents [4]. Benzothiazoles are one such type of compound, whose core molecular structure can be found in a large number of natural products [5]. The benzathiazoles exhibit great pharmaceutical importance due to their potent biological activities, these include anti-bacterial [6], anti-ulcer [7], anti-cancer [8], anti-inflammatory [9] and anti-viral [10] to mention a few.

Inspired by the aforementioned medicinal activity of the benzothiazoles, we have set out to create two small libraries of structurally similar *N*-alkylated molecules: 2,3,3-trimethylindolenine (**1a**–**m**) and 2-methylbenzothiazole (**2a**–**m**). Each of these subclasses has a step-wise increase in linear alkyl chain length (C1–C10). The resulting derivatives show varying degrees of lipophilicity, as judged by their log *P* (base 10 logarithm of the partition coefficient) values (shown in Tables 1 and 2, respectively). It's widely accepted that an increase in the length of the carbon

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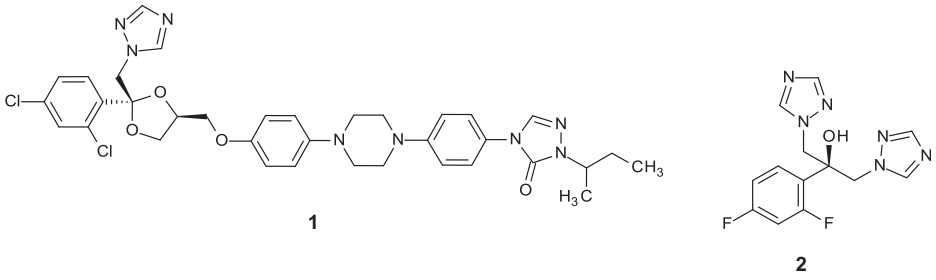


Fig. 1. Two licensed triazole antifungals, Itraconazole **1** and Fluconazole **2**.

chain, goes hand in hand with an increase in pharmacological activity (this is usual from C1–C10), due to an increase in lipid membrane penetration. However, above C10 a decrease in activity is usually seen and this is attributed to poor transport through aqueous media [10]. Our intention was to examine the growth inhibition of three yeast strains up to a linear chain length of C10 and assess any potential antifungal activity observed.

The compounds were tested against three different yeast species; *Schizosaccharomyces pombe* (*S. pombe*), a fission yeast; *Saccharomyces cerevisiae* (*S. cerevisiae*), a budding yeast and *Candida albicans* (*C. albicans*) a diploid fungus known for opportunistic oral and genital infections in humans [11]. Both *S. pombe* and *S. cerevisiae* yeast species are used extensively in eukaryotic microbiological research and demonstrate close homology to a number of pathogenic fungi. For example, *S. cerevisiae* is closely related to *C. albicans*, which, as mentioned above, is a widespread commensal and important pathogen of humans. Similarly, *S. pombe* is closely related to *Pneumocystis jiroveci*, a yeast-like fungus which commonly causes pneumonia in immunocompromised patients [12]. *S. cerevisiae* is of particular importance, emerging as an opportunistic pathogen, especially in immunosuppressed and immunocompromised patients, and has been associated with fungemia, endocarditis, peritonitis, meningitis, ventriculitis, and with polymicrobial fatal pneumonia in HIV/AIDS patients [13–19]. These yeast species can serve as excellent models to learn more about pathogenic fungi, in particular with regard to regulatory features and drug therapy, because they share many characteristics with their pathogenic relatives [20–22].

2. Results and discussion

2.1. Chemistry

The synthesis of both sets of salts was straightforward and required no harsh or unusual synthetic methodologies, as shown in Scheme 1. The salts of 2,3,3-trimethylindolenine and 2-methylbenzothiazole were prepared by alkylation with the corresponding alkyl/benzyl halides or sultones to afford the compounds in excellent to poor yield as shown in Tables 1 and 2. Compounds **1a**–**d**, **k**–**m** and **2a**–**m** precipitated from the reaction mixture and were isolated by filtration under reduced pressure. What is noticeable is the increased hygroscopic nature of the indolenium bromide salts with increased chain length (C5–C10). To counteract this problem, a counter ion exchange based on the Finkelstein reaction was performed to yield the iodide salts which are not as hygroscopic as the bromides; thus easier to handle and work with from a biological viewpoint. The synthesis of four 2,3,3-trimethylindolenine salts (**1a**, **e**, **k** and **l**) are shown in the experimental section below for clarity. The synthesis of the full set of compounds (**1a**–**m** and **2a**–**m**) is also shown in the Supporting information.

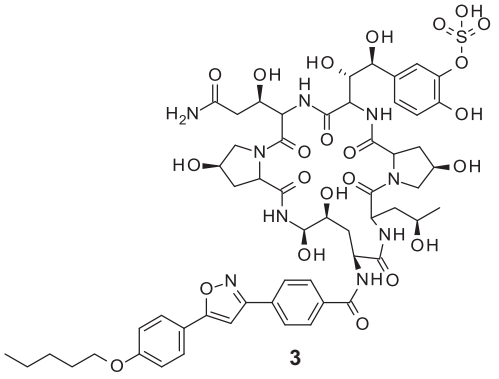
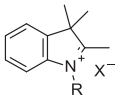


Fig. 2. Micafungin **3**, an echinocandin natural product antifungal.

2.2. Antifungal activity

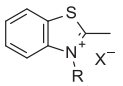
The synthesised compounds were tested *in vitro* to determine growth inhibitory activity. Minimum inhibitory concentration (MIC) values were determined in sets, by comparison with 2,3,3-trimethylindolenine (**1n**) and 2-methylbenzathiazole (**2n**) (purchased from Alfa Aesar), under the same conditions noted in

Table 1
The antifungal data for compounds **1a**–**n**.

Number				MIC (μg/mL)		
	R	% Yield	Log *P*	Sc	Sp	Ca
1a	CH_3	58	−0.691	500	500	1000
1b	CH_2CH_3	86	−0.315	500	500	1000
1c	$CH_2CH_2CH_3$	85	0.188	250	250	1000
1d	$CH_2(CH_2)_2CH_3$	67	0.747	250	250	1000
1e	$CH_2(CH_2)_3CH_3$	38 (99)	1.252	250	125	1000
1f	$CH_2(CH_2)_4CH_3$	22 (99)	1.758	125	125	1000
1g	$CH_2(CH_2)_5CH_3$	19 (86)	2.263	125	62.5	1000
1h	$CH_2(CH_2)_6CH_3$	11 (40)	2.768	62.5	62.5	1000
1i	$CH_2(CH_2)_7CH_3$	20 (27)	3.273	31.3	31.3	1000
1j	$CH_2(CH_2)_8CH_3$	53 (99)	3.778	15.6	15.6	500
1k	$CH_2C_6H_5$	77	0.904	1000	1000	1000
1l	$CH_2CH_2CH_2SO_3^-$	58	−4.33	1000	1000	1000
1m	$CH_2CH_2CH_2CH_2SO_3^-$	50	−4.114	1000	1000	1000
1n	NA	–	3.286	1000	250	500

Minimal Inhibitory Growth Concentration (MIC) of synthesised compounds tested in *S. cerevisiae*, *S. pombe*, and *C. albicans*. Cells were inoculated at a concentration of 3×10^4/ml. Culture media tested were in yeast extract broth (YE) for *S. pombe* and complex growth media (YPD) *for S. cerevisiae and C. albicans*. Growth of yeast was determined visually after 24 h incubation at 30 °C. The MIC of the compounds was determined to be the well before yeast growth was first seen. The experiment was repeated twice. Sc – (*S. cerevisiae*), Sp – (*S. pombe)* and Ca – (*C. albicans*). Yielded brackets highlight yield for iodo counter ion exchange. X^- Indicates either a bromide or iodide counter ion.

Table 2
The antifungal data for compounds **2a**–**n**.

Number				MIC (μg/mL)		
	R	% Yield	Log *P*	Sc	Sp	Ca
2a	CH_3	87	−1.077	500	500	1000
2b	CH_2CH_3	41	−0.701	500	250	1000
2c	$CH_2CH_2CH_3$	44	−0.199	250	250	1000
2d	$CH_2(CH_2)_2CH_3$	51	0.36	250	250	1000
2e	$CH_2(CH_2)_3CH_3$	11	1.252	62.5	250	1000
2f	$CH_2(CH_2)_4CH_3$	7	1.371	62.5	250	1000
2g	$CH_2(CH_2)_5CH_3$	10	1.876	31.3	125	1000
2h	$CH_2(CH_2)_6CH_3$	15	2.381	15.6	62.5	1000
2i	$CH_2(CH_2)_7CH_3$	9	2.887	7.8	31.3	500
2j	$CH_2(CH_2)_8CH_3$	6	3.392	3.9	7.8	250
2k	$CH_2C_6H_5$	90	0.517	1000	1000	1000
2l	$CH_2CH_2CH_2SO_3^-$	50	−4.576	1000	1000	1000
2m	$CH_2CH_2CH_2CH_2SO_3^-$	7	−4.411	1000	1000	1000
2n	N/A	–	2.085	1000	250	1000

Minimal Inhibitory Growth Concentration (MIC) of synthesised compounds tested in *S. cerevisiae*, *S. pombe*, and *C. albicans*. Cells were inoculated at a concentration of 3×10^4/ml. Culture media tested were in yeast extract broth (YE) for *S. pombe* and complex growth media (YPD) *for S. cerevisiae and C. albicans*. Growth of yeast was determined visually after 24 h incubation at 30 °C. The MIC of the compounds was determined to be the well before yeast growth was first seen. The experiment was repeated twice. Sc – (*S. cerevisiae*), Sp – (*S. pombe)* and Ca – (*C. albicans*). X^- Indicates either a bromide or iodide counter ion.

Tables 1 and 2. It is important to note that the yeast cell surfaces carry a negative charge [23] and thus a good interaction between the yeast cell surface should be observed with the quaternary *N*-alkylated compounds (**1a**–**m** and **2a**–**m**).

We chose to vary one physiochemical property (log *P*) to examine how this affected growth inhibition (log 1/*C*), due to the medicinal target i.e. yeast cell wall. Using a virtual method, the log *P* (the base 10 logarithm was used throughout) values were obtained [24]. Neither sets of compounds inhibited the growth of *C. albicans*, so this species was removed from subsequent analyses.

From analysing the MIC values in Tables 1 and 2, the assumption can be made that the 2,3,3-trimethylindolenine salts (**1a**–**h**) showed a poor growth inhibition relationship against both the *S. cerevisiae* and *S. pombe* yeast strains. However, compounds **1i**–**j** with an *N*-alkylated chain length > C9, are shown to be the most potent of the set. The lowest MIC value was recorded against both the aforementioned stains at 15.6 μg/mL for compound **1j**. In comparison the 2-methylbenzathiazole salts (**2a**–**j**) showed varying degrees of growth inhibition. Against *S. cerevisiae*, compound **2a**–**f** showed poor growth inhibition. Conversely, compounds **2g**–**j** with *N*-alkylated chain length > C7, gave the lowest MIC values for growth inhibition, with the compound **2j** displaying most potency at 3.9 μg/mL. However, upon comparison with the *S. pombe* yeast strain, a different result is observed. Compounds **2a**–**h** are deemed to be the least potent with the highest MIC values, yet compounds **2i**–**j** with *N*-alkylated chain length > C9 show the lowest MIC values, again with compound **2j** displaying most potency at 7.8 μg/mL.

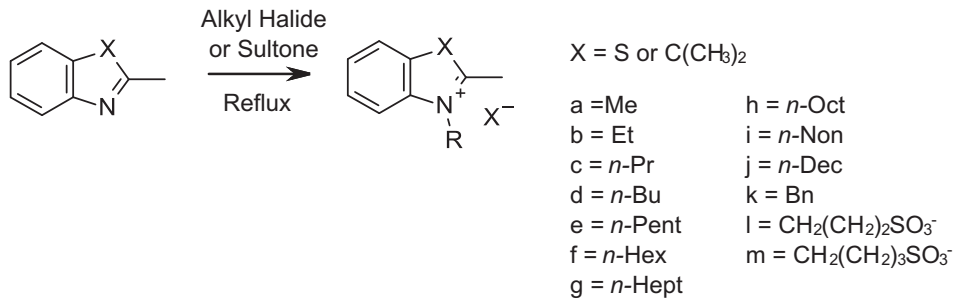
It is also noted that the sulfonic acid salts of both the 2,3,3-trimethylindolenine and the 2-methylbenzothiazole (**1l**–**m** and **2l**–**m**) showed no antifungal activity, it is assumed that the presence of the sulfonic acid groups tends to increase their solubility and reduces their growth inhibitory characteristics, possibly through suppressing membrane permeability and cellular uptake. It is also noted that the benzyl substituent showed no activity against any of the three fungi types and again this could possibly be due to the molecule having little membrane permeability due to the sp^2 hybridised ring system. It is interesting to note that the starting material 2,3,3-trimethylindolenine (**1n**) showed slight growth inhibition of *S. pombe* and *C. albicans* with MIC's of 250 μg/mL and 500 μg/mL respectively. The 2-methylbenzathiazole (**2n**) also showed growth inhibition of *S. pombe* again at 250 μg/mL but showed no growth inhibition against *S. cerevisiae* or *C. albicans*. This indicates that varying the alkyl chain length has a very positive impact on growth inhibition.

2.2.1. Control bioassays

Two bioassays with Amphotericin B **4** and Thiabendazole **5** (Fig. 4) were set up to compare the efficacy of our range of *N*-alkylated compounds against classic antifungal agents. The rationale behind the choice of these antifungals was as follows. Amphotericin B is an extremely potent antifungal and targets the membrane sterol of fungal cell membranes, this choice of antifungal agent is crucial as it provides a similar mode of action in comparison to our compounds. Thiabendazole inhibits nucleic acid metabolism and protein synthesis and has a similar core structure when compared against our compounds. However, the Thiabendazole has previously shown poor activity against *C. albicans* [25] and *S. cerevisiae*, which may be explained by poor aqueous solubility [26]. Amphotericin B showed high potency against all three species of yeast at 0.49 μg/mL, which is in line with published data [25]. Thiabendazole, showed poor results against *S. cerevisiae* and *C. albicans* species at 1000 μg/mL, however against *S. pombe* species the MIC was 31.25 μg/mL. We thus conclude that the longer chain *N*-alkylated 2,3,3-trimethylindolenines and 2-methylbenzothiazoles (**1i**–**j** and **2g**–**j**) at concentrations <35 μg/mL show a comparable efficacy profile with the known antifungals used in the treatment of fungal infections. This suggests further investigation into these compounds would be beneficial for the treatment of fungal infections. The bioassays were set-up in line with experimental procedure highlighted in Section 4.2.

2.3. Statistical analysis

The intention was to analyse the relationship between increasing chain length and growth inhibition by ordinary least squares



Scheme 1. The synthetic strategy to making the 2,3,3-trimethylindolenine (**1a**–**m**) and 2-methylbenzothiazole (**2a**–**m**) salts.

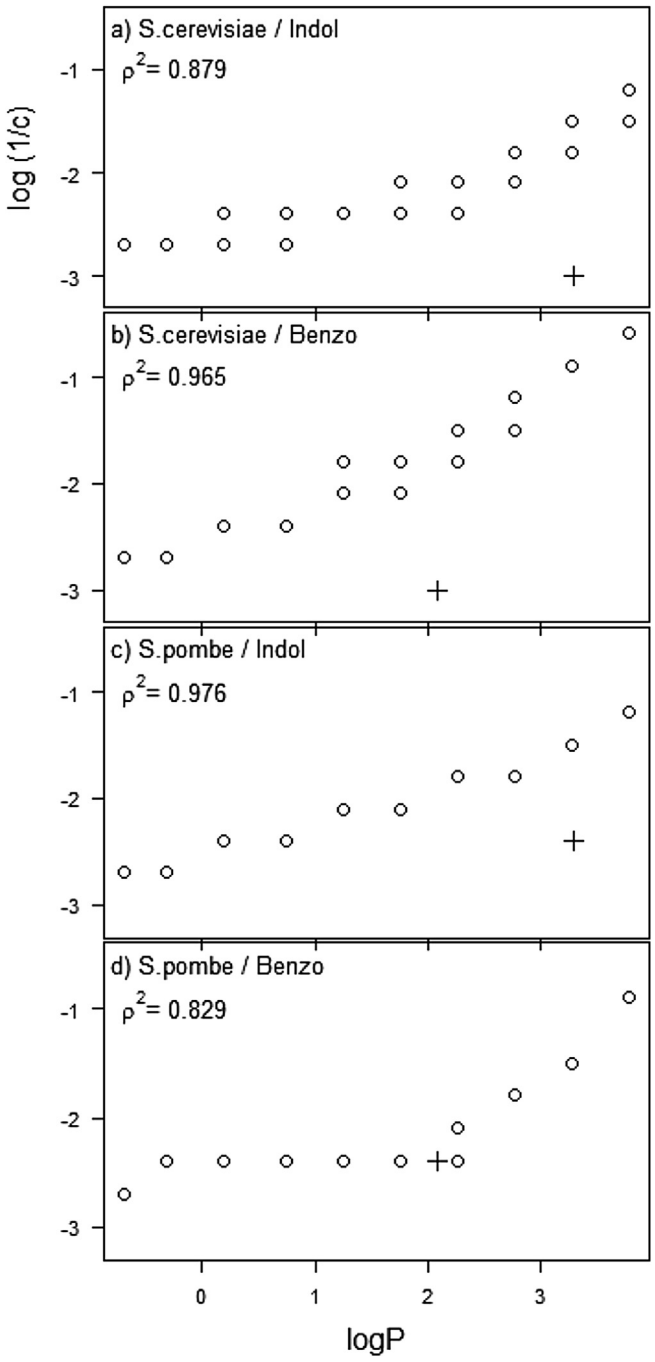


Fig. 3. Relationship between (base 10 logarithms of) the inverse of minimum concentration for growth inhibition and the partition coefficient for the fungal species *S. pombe* and *S. cerevisiae* and alkylated molecule type. Two replicates were used, and points overlap where only one appears at each log *P* value. 2,3,3-Trimethylindolenine (Indol) (**1a**–**j**) and 2-methylbenzathiazole (Benzo) (**2a**–**j**) values for each replicate are marked by a plus symbol. The square of Spearman's rho (see text for explanation) on each plot quantifies the strength of the relationship.

regression, since, with the limited range of log *P* values, a straight line relationship was expected. However, the relationship was not sufficiently linear to use this method, as a slight upward curve – made more noticeable in a plot of residuals against fitted values (not shown) – is evident (Fig. 3a–d). Furthermore, since the dependent variable was restricted by the small numbers of serial dilution concentrations, consistent with experimental protocol, residual values from the model were very unlikely to be normally distributed. For these reasons linear regression was deemed inappropriate, and a correlation was used. This is unfortunate as it means the relationship between the variables could not be quantified as a straight line equation. As the log (1/*C*) variable was not normally distributed in three out of four cases, a non-parametric method was required. Spearman's rank correlation coefficient is simply Pearson's Product–Moment correlation on ranks rather than original values. Its statistic ρ ('Spearman's rho') is thus related to r^2, the coefficient of determination, so values of ρ^2 are given in Fig. 3 to facilitate comparison with studies which report this quantity.

A strong relationship is clear in all four plots of Fig. 3, with that for *S. pombe* and 2-methylbenzothiazole (Fig. 3d) the weakest ($\rho = 0.910$, $t = 9.34$, $n = 20$, $p < 0.0001$); increasing the alkyl chain length increases growth inhibition.

3. Conclusion

The quaternary *N*-alkylated derivatives of both 2,3,3-trimethylindolenine and 2-methylbenzothiazole salts show varying degrees of antifungal activity with the longer chain substituents being more potent against *S. cerevisiae* and *S. pombe*. We propose that the growth inhibition can be attributed to the charged *N*-alkylated compounds with the increasing linear chain lengths. We postulate that these compounds are attracted to the negatively charged yeast membrane, with the longer lipophilic chains being absorbed into and subsequently distorting the lipid bilayer. Compounds **1i**–**j** and **2g**–**j**, all show MIC values of <35 μg/mL and are thus deemed from this study to be the most potent towards *S. cerevisiae* and *S. pombe*. Together with further compounds, which are being generated with higher degrees of lipophilicity these compounds will be taken forward for mammalian screening. This preliminary study has also highlighted that none of the synthesised compounds (**1a**–**m** and **2a**–**m**) has shown any real activity towards *C. albicans*. This response may be the result of *C. albicans*' ability to adapt to the antifungal stress and develop drug tolerance. As a diploid fungus, *C. albicans* contains a large number of drug exclusion mechanisms with varying substrate specificities enabling it to obtain tolerance to many novel antifungals, a resistance not seen with the haploid yeast species, *S. cerevisiae* and *S. pombe* [27,28]. The results highlighted above imply that longer *N*-alkylated (*i.e.* >*C*7) 2,3,3-trimethylindolenines and 2-methylbenzothiazole salts show potential for selective targeting towards *S. cerevisiae* infections which are a major problem in health care.

4. Experimental section

4.1. General procedure

^{1}H and ^{13}C NMR spectra were measured on either a Bruker DPX 250 MHz, Bruker Avance-III 300 MHz or a Bruker Avance 400 MHz spectrometer at ambient temperature with tetramethylsilane (TMS) as internal standard for ^{1}H NMR and deuteriochloroform ($CDCl_3$, δ_C 77.23 ppm) and deuteriodimethylsulfoxide (d_6-DMSO, δ_C 39.51 ppm) for ^{13}C NMR unless otherwise stated. All chemical shifts are quoted in δ (ppm) and coupling constants in Hertz (Hz) using the high frequency positive convention. The abbreviations used for the multiplicity of the NMR signals are: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sex = sextet, m = multiplet, dd = doublet of doublet, td = triplet of doublets, dm = doublet of multiplets, br s = broad singlet, etc. Mass spectra were recorded on a Thermo Scientific Trace LC Ultra DSQ II using Electron Ionisation (LCMS-EI). Infrared spectra were recorded on a Specac ATR with a He Ne –633 nm laser. Thin Layer Chromatography (TLC) was carried out on Machery–Nagel polygramSil/G/UV_{254} pre-coated plates. Melting point (Mp) analysis was carried out using the Griffin

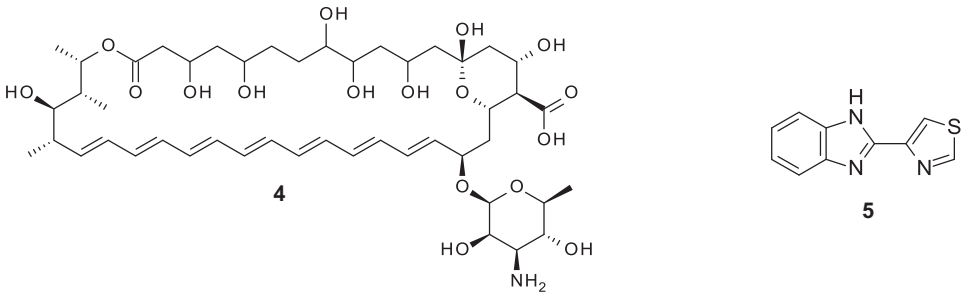


Fig. 4. The structures of Amphotericin B **4** and Thiabendazole **5**.

melting point apparatus. All chemicals were purchased from Sigma–Aldrich or Alfa Aesar and used without purification.

*4.1.1. 1,2,3,3-Tetramethyl-3H-indol-1-ium iodide (**1a**)*

2,3,3-Trimethylindolenine (63.0 mmol) was dissolved in iodomethane (168 mmol) and with constant stirring, the solution was refluxed for 24 h. The precipitate produced was filtered under suction, washed with *n*-hexane and dried *in vacuo* to yield the product (58%) as a pink solid.

^{1}H NMR (d_6-DMSO, 300 MHz): δ 7.90 (t, J = 6.0 Hz, 1H, Ar–H), 7.82 (t, J = 6.0 Hz, 1H, Ar–H), 7.66–7.61 (m, 2H, Ar–H), 3.96 (s, 3H, N–C$\underline{H}_3$), 2.75 (s, 3H, C–C$\underline{H}_3$), 1.52 (s, 6H, C–(C$\underline{H}_3$)$_2$). ^{13}C NMR (d_6-DMSO, 75.4 MHz): δ 196.45, 142.56, 142.05, 129.77, 129.28, 123.76, 115.57, 100.00, 54.38, 22.14, 14.51. IR (ATR, cm^{-1}): 2968.4, 1628.9, 1455.1, 1392.7, 1357.8, 774.4. MS (ESI) *m*/*z*: 174.09 [M$^+$]. Melting point = 255–257 °C.

*4.1.2. 1-Pentyl-2,3,3-trimethyl-3H-indol-1-ium iodide (**1e**)*

2,3,3-Trimethylindolenine (15.0 mmol) was dissolved in acetonitrile (10.0 mL), followed by the addition of 1-bromopentane (20.0 mmol). With constant stirring, the solution was refluxed for 24 h to produce a brown solution. The solution was concentrated under reduced pressure to yield brown oil, which was purified by column chromatography to yield the product as a bromide salt (38%) which was hygroscopic. The bromide salt (1.00 mmol) was dissolved in acetone (10.0 mL) and heated under reflux with sodium iodide (1.00 mmol) for 24 h. The white solid produced (KBr) was filtered and the solution was evaporated under reduced pressure yielding the iodide salt (99%) as a purple solid.

^{1}H NMR (d_6-DMSO, 300 MHz): δ 7.97 (t, J = 6.0 Hz, 1H, Ar–H), 7.84 (t, J = 6.0 Hz, 1H, Ar–H), 7.64–7.61 (m, 2H, Ar–H), 4.44 (t, J = 9.0 Hz, 2H, N–C$\underline{H}_2$), 2.83 (s, 3H, C–C$\underline{H}_3$), 1.82 (sex, J = 9.0 Hz, 2H, CH$_2$–C$\underline{H}_2$–CH$_3$), 1.53 (s, 6H, C–(C$\underline{H}_3$)$_2$), 1.38–1.31 (m, 4H, C–C$\underline{H}_2$), 0.88 (t, J = 6.0 Hz, 3H, CH$_2$–C$\underline{H}_3$). ^{13}C NMR (d_6-DMSO, 75.4 MHz): δ 196.65, 142.22, 141.34, 129.83, 129.40, 124.20, 105.20, 54.70, 28.42, 27.47, 26.85, 22.61, 22.18, 15.91, 10.33. IR (ATR, cm^{-1}): 3412.6, 3213.4, 1605.1. MS (ESI) *m*/*z*: 230.20 [M$^+$]. Melting point = 139–140 °C.

*4.1.3. 1-Benzyl-2,3,3-trimethyl-3H-indol-1-ium bromide (**1k**)*

Benzyl bromide (6.80 mmol) dissolved in acetonitrile (20.0 mL) was heated with constant stirring until a state of reflux was established. 2,3,3-Trimethylindolenine (6.30 mmol) dissolved in acetonitrile (20.0 mL) was added dropwise to the reaction mixture from a dropping funnel. Once all the reactants had been added the reaction was continued for 48 h. The precipitate produced was filtered under suction, washed with *n*-hexane and dried *in vacuo* to yield the product (77%) as a red hygroscopic solid.

^{1}H NMR (d_6-DMSO, 250 MHz): δ 7.89 (d, J = 7.0 Hz, 1H, Ar–H), 7.84 (d, J = 7.0 Hz, 1H, Ar–H), 7.67–7.59 (m, 2H, Ar–H), 7.45–7.38 (m, 5H, Ar–H), 5.87 (s, 2H, N–CH$_2$), 3.59 (s, 3H, N–C–CH$_3$), 1.55 (s, 6H C–(CH$_3$)$_2$). ^{13}C NMR (d_6-DMSO, 75.4 MHz): δ 198.7, 142.5, 141.6, 132.7, 130.1, 129.5, 129.3, 128.1, 124.2, 116.5, 55.1, 51.2, 15.1, 9.81. IR (ATR, cm^{-1}): 2969, 1603, 1454, 931, 741, 701, 567. MS (ESI) *m*/*z*: 250.33 [M$^+$]. Melting point = 226–230 °C.

*4.1.4. 2,3,3-Trimethyl-1-(3-sulfonatopropyl)-3H-indol-1-ium (**1l**)*

To a solution of 2,3,3-trimethylindolenine (62.3 mmol) in toluene (50.0 mL) was added 1,3-propanesultone (93.5 mmol) and with constant stirring, the solution was refluxed for 24 h. The precipitate produced was filtered under suction, washed with toluene and dried *in vacuo* to yield the product (58%) as a white solid.

^{1}H NMR (d_6-DMSO, 300 MHz): δ 7.43 (d, J = 9.0 Hz, 2H, Ar–H), 7.88 (t, J = 9.0 Hz, 1H, Ar–H), 7.78 (t, J = 9.0 Hz, 2H, Ar–H) 4.91 (t, J = 9.0 Hz, 2H, N–C$\underline{H}_2$) 3.54 (s, 6H, C–(C$\underline{H}_3$)$_2$), 2.65 (t, J = 6.0 Hz, 2H, CH$_2$–C$\underline{H}_2$) 2.16 (quin, J = 6.0 Hz, 2H, CH$_2$–C$\underline{H}_2$–C$\underline{H}_2$) 2.08 (s, 3H, C–C$\underline{H}_3$). ^{13}C NMR (d_6-DMSO, 75.4 MHz) δ 207.08, 177.75, 177.72, 141.35, 129.61, 128.51, 125.01, 117.31, 47.80, 47.78, 31.17, 24.72, 17.21 IR (ATR, cm^{-1}): 2904.9, 1634.1, 1455.5, 1326.4, 1161.7, 1028.6, 781.9. MS (ESI) *m*/*z*: 282.32 [M$^+$] Melting point = 265–268 °C.

4.2. Determination of antifungal activity

The growth inhibitory activity of the compounds (**1a**–**n** and **2a**–**n**) were determined by screening *S. pombe*, *S. cerevisiae and C. albicans* using the following method:

Yeast species were inoculated into relevant media; *S. pombe* (NJ2 *h*$^-$ *ura4-D18 leu1-32 ade6-M210 his7-366*) [29] into yeast extract broth (YE) [30], and *S. cerevisiae* (strain BY4741a, a derivative of S288C), (*MAT***a***his*3*1 leu2*0 *met15*0 *ura3*0) [31] and *C. albicans* (strain SC5314) [32] into complex media (YPD) [33]. The culture was then incubated for 12 h at 30 °C with shaking at 200 rpm. Stock solutions of the compounds were prepared in 20% (v/v) DMSO and culture media. DMSO and culture media were all used as controls for the experiment. 3×10^4 yeast cells were transferred into the wells of a 96-well plate. A 1:2 serial dilution of the compounds was then performed. The wells were inspected visually for growth of yeast after 24 h of incubation at 30 °C. Growth was indicated by full or partial white appearance of yeast on the bottom of the wells. The MIC values of the compounds were determined to be the well before yeast growth was first seen. The experiment was repeated two times to ensure reproducibility of the results.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.031.

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