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1           **Chemoenzymatic synthesis, nanotization and anti-*Aspergillus***  
2                           **activity of optically enriched fluconazole analogues**

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23 **Running title:** Chemoenzymatic synthesis of optically enriched fluconazole  
24 analogues.

25 **Keywords:** chemoenzymatic synthesis, fluconazole, antifungal agents

26

27 **Abstract**

28 Despite recent advances in diagnostic and therapeutic advances in antifungal  
29 research, aspergillosis still remains a leading cause of morbidity and mortality. One  
30 strategy to address this problem is to enhance the activity spectrum of known  
31 antifungals, and we now report the first successful application of *Candida antarctica*  
32 lipase (CAL) for the preparation of optically enriched fluconazole analogs. Anti-  
33 *Aspergillus* activity was observed for an optically enriched derivative, (-)-*S*-2-(2',4'-  
34 difluorophenyl)-1-hexyl-amino-3-(1''',2''',4''') triazol-1'''-yl-propan-2-ol, which exhibits  
35 MIC values of 15.6 µg/mL and 7.8 µg/disc in microbroth dilution and disc diffusion  
36 assays, respectively. This compound is tolerated by mammalian erythrocytes and  
37 cell lines (A549 and U87) at concentrations of up to 1000 µg/mL. When incorporated  
38 into dextran nanoparticles, the novel, optically enriched fluconazole analog exhibited  
39 improved antifungal activity against *Aspergillus fumigatus* (MIC = 1.63 µg/mL).  
40 These results not only demonstrate the ability of biocatalytic approaches to yield  
41 novel, optically enriched fluconazole derivatives but also suggest that  
42 enantiomerically pure fluconazole derivatives, and their nanotised counterparts,  
43 exhibiting anti-*Aspergillus* activity may have reduced toxicity.

44 Aspergillosis remains a significant threat to public health, and, in spite of  
45 continuous efforts to improve timely diagnosis and clinical therapies, mortality  
46 caused by this disease remains unacceptably high [1, 2]. Current therapeutic  
47 options for treating *Aspergillus*-induced disorders include antifungal agents  
48 such as polyenes, azoles and echinocandins [3, 4]. Thus the discovery of new  
49 antifungal compounds remains important given the need to address the  
50 development of drug resistance in pathogenic fungi [5-7]. One approach to  
51 accomplishing this goal is to prepare new derivatives of existing drugs with  
52 broad spectrum activity and enhanced pharmacokinetic properties. As part of  
53 our on-going efforts to use lipases [8-12], which catalyze reactions with high  
54 degree of chemo-, regio- and stereoselectivity in organic synthesis, we  
55 became interested in preparing new antifungals using biocatalysis.

56 Fluconazole, introduced in 1990, is a bis-triazole antifungal drug which  
57 possesses interesting pharmacokinetic properties, such as low plasma binding  
58 affinity, good water solubility, low first pass metabolism, high oral  
59 bioavailability and a long half-life, all of which should make it a drug of choice  
60 for treating fungal infections [13, 14]. On the other hand, fluconazole has been  
61 reported to exhibit only limited activity against *Aspergillus* infections [15],  
62 which has led to many reports concerning the synthesis of various types of  
63 fluconazole derivatives and their chiral separation/resolution into constituent  
64 enantiomers [16-19]. We now report the use of *Candida antarctica* lipase  
65 (CAL-B) in catalysing the addition of amines to an achiral epoxide to yield  
66 optically enriched fluconazole analogues in which one of the triazole rings is  
67 replaced by *n*-alkylamino and cycloalkylamino substituents. To the best of our  
68 knowledge, the work reported herein is the first direct synthesis of optically

69 enriched fluconazole analogues using biocatalytic methods. *In vitro* assays  
70 show that the optically enriched analogues exhibit more potent antifungal  
71 activity than the corresponding racemic mixtures. Interestingly, this bioactivity  
72 can be enhanced by their encapsulation in dextran-based nanoparticles [20].

73

## 74 Results

75 **Synthesis of fluconazole analogues.** A series of linear and cyclic alkylamines  
76 was screened for reaction with the epoxide ring of ( $\pm$ )-1-[2-(2, 4-difluorophenyl)-  
77 oxiranylmethyl]-1*H*-[1,2,4]-triazole (**1**, Figure 1) in a number of different organic  
78 solvents. Three different immobilized lipases were also evaluated for their ability to  
79 catalyze this reaction: *Candida rugosa* lipase (CRL), porcine pancreatic lipase (PPL)  
80 and CAL-B. Although the ring-opening reactions catalysed by CRL and PPL were of  
81 no practical utility, when the reaction was performed in the presence of CAL-B in  
82 tetrahydrofuran (THF) as solvent, the desired products (**3a-j**, **5a** and **5b**) were  
83 obtained with good yields in optically enriched forms (Figure 1, and Tables S1 and  
84 S2 in Supporting Information). Very importantly, all of the twelve novel fluconazole  
85 analogues formed in the lipase-catalyzed reactions were optically active showing  
86 that aminolysis of the racemic starting epoxide ( $\pm$ )-1 had proceeded in an  
87 enantioselective fashion (Table S1). These twelve compounds could also be  
88 prepared in racemic form, as viscous oils in 75-80 % yields, by direct reaction of the  
89 alkylamines with the racemic epoxide precursor ( $\pm$ )-1 in THF at 55 °C. The time  
90 taken for complete consumption of aliphatic amines **2a-j**, **4a** and **4b** in the CAL-B  
91 catalyzed reaction varied between 18h and 28h, which was considerably shorter  
92 than the 48-56 h required for the chemical addition of the amines (Table S2 in  
93 Supporting Information). The structures of all twelve fluconazole analogues were

94 unambiguously established on the basis of spectroscopic data (IR,  $^1\text{H}$ - and  $^{13}\text{C}$  NMR,  
95 and mass spectra), and by comparison to literature data for known compounds **3b**,  
96 **3c**, **5a** and **5b** [21, 22].

97 Although the enantiomeric enrichment of the fluconazole analogues prepared  
98 by lipase-catalyzed addition was not established, we were able to assign the  
99 absolute configuration of the major enantiomer using the optical activity of the  
100 unreacted epoxide isolated from the reaction mixture. These samples rotated  
101 polarized light in a positive (+) direction, meaning that the recovered, unreacted  
102 epoxide was enriched in the enantiomer for which the stereogenic centre has the (S)  
103 configuration (Table S1 in Supporting Information) [23]. CAL-B therefore  
104 preferentially employs (-)-*R*-**1** in the aminolysis reaction and, assuming a standard  
105  $\text{S}_{\text{N}}2$  mechanism for reaction of the amine with the epoxide, we can deduce that the  
106 fluconazole analogues must be enriched in the (-)-*S*-enantiomer (Figure 1).

107  
108 **Antifungal activities of the fluconazole analogs** Pathogenic *Aspergillus*  
109 strains (*Aspergillus fumigatus* ITCC 6604, *Aspergillus flavus* ITCC 5192, and  
110 *Aspergillus niger* ITCC 0004) were used to determine the *in vitro* antifungal efficacy  
111 of the fluconazole analogues, in both their optically enriched and racemic forms.  
112 These experiments used standard microbroth dilution (MDA), disc diffusion (DDA)  
113 and spore germination inhibition (PSGI) assays [24, 25]. We note that the MDA  
114 assay is based on the same basic principle as that used in the CLSI micro-dilution  
115 protocol. The only difference between the two assays is that CLSI uses RPMI  
116 medium to prepare diluted drug solutions rather than the Sabouraud dextrose broth  
117 (a medium used to culture *Aspergillus* in the laboratory) used by us to determine the  
118 MIC of the fluconazole derivatives. As recommended in CLSI protocols, we carefully

119 monitored MDA parameters with respect to preparation of the test compounds,  
120 medium preparation, temperature, inoculum size, incubation time, minimum  
121 inhibitory concentration (MIC)/endpoint determination, data recording and  
122 interpretation of results to ensure the validity and quality of our results. On this point,  
123 we note that a previous study from our laboratory [26] showed that results with RPMI  
124 1640 or RPMI 1640 containing glucose were not different from those obtained by  
125 using Sabouraud dextrose broth.

126 On the basis of their MIC values, all the compounds exhibited moderate to  
127 good anti-*Aspergillus* activities, with the analogue (-)-**S-3d** being more potent than  
128 the commercially available fluconazole (Table 1). We also observed that optically  
129 enriched mixtures of (-)-**S-3a**, (-)-**S-3c**, (-)-**S-3d**, (-)-**S-3e** and (-)-**S-5b** were more  
130 active than the corresponding racemates. These data also confirm that introducing a  
131 linear aliphatic alkyl side chain is important for imparting antifungal activity, as  
132 reported previously [23, 24]. On the other hand, when additional, “distal” N-  
133 substituted alkyl groups were present, as in compounds (-)-**S-3g** and (-)-**S-3h**,  
134 antifungal activity was completely lost (Table 1). Compounds **3j** and **5a** exhibited no  
135 biological activity in microbroth dilution assays and were not studied further. Our  
136 work also shows that the length of the alkyl side chain is an important factor in  
137 determining activity, i.e. the compound (-)-**S-3d**, containing an n-hexyl moiety, has  
138 higher activity than (-)-**S-3a**, (-)-**S-3b** and (-)-**S-3c**, which contain ethyl, *n*-propyl and  
139 *n*-butyl groups, respectively (Table 1). Decreasing the linker chain length also led to  
140 higher activity. Optically enriched (-)-**S-3d** was the most potent compound against  
141 *Aspergillus fumigatus* (Table 1) and was therefore used to examine how  
142 encapsulation in dextran nanoparticles might impact anti-fungal activity.

143

144 **Characterization of (-)-S-3d release from O-alkylated dextran**  
145 **nanoparticles.** Dextran nanoparticle-based drug delivery systems are  
146 biocompatible, biodegradable, possess low immunogenicity [20], and can be used  
147 for controlled release of pharmacologically active substances [27]. We therefore  
148 encapsulated optically enriched (-)-S-3d into three types of dextran nanoparticles,  
149 derivatized with O-hexadecyl, O-decyl and O-heptyl chains to ensure amphiphilicity,  
150 and examined their effect on anti-*Aspergillus* activity. After trapping (-)-S-3d within  
151 each of the nanoparticles by self-assembly (encapsulation efficiencies for the O-  
152 hexadecyl, O-decyl and O-heptyl nanoparticles were  $50 \pm 4$  %,  $22 \pm 2$  % and  $30 \pm 2$   
153 %), the resulting particle size distributions were determined using dynamic light  
154 scattering (DSL). These measurements showed that the sizes of the O-hexadecyl-,  
155 O-decyl- and O-heptyl- derivatized nanoparticles were  $140 \pm 16$  nm,  $187 \pm 13.16$  nm  
156 and  $183 \pm 14.73$  nm, respectively, and that all of the samples had a low  
157 polydispersity index ( $< 0.3$ ) (Supporting Information). Examination of the rate at  
158 which the fluconazole analogue (-)-S-3d was released from each of the three types  
159 of nanoparticles, showed an initial burst for the O-hexadecyl- and O-decyl-  
160 derivatized nanoparticles (Figure 2).

161

162 **Anti-*Aspergillus* activity and cytotoxicity of (-)-S-3d encapsulated in O-**  
163 **alkylated dextran nanoparticles.** We next examined the effect of nanoparticle  
164 encapsulation on the activity of (-)-S-3d against *Aspergillus fumigatus* using a  
165 microbroth dilution assay (Figure 3). After 48 h of incubation (approximately 80 %  
166 release), (-)-S-3d encapsulated in O-decyl-derivatized nanoparticles inhibited the  
167 growth of *Aspergillus fumigatus* at an effective concentration of  $3.16 \mu\text{g/mL}$ . Perhaps  
168 more importantly, when the optically enriched fluconazole analogue was



169 encapsulated in *O*-hexadecyl nanoparticles, complete inhibition of *Aspergillus*  
170 *fumigatus* growth was achieved at an effective concentration of 1.63 µg/mL (41.3 %  
171 release at an initial concentration of 3.95 µg/mL). In addition, nanoparticle-  
172 encapsulated (-)-**S-3d** exhibits activity at a lower concentration when compared to  
173 both fluconazole and free (-)-**S-3d**. Although we believe that this effect is associated  
174 with sustained release of the compound over time, it is also possible that drug  
175 uptake is more efficient because the drug in its encapsulated form is more efficiently  
176 captured by the cells. The general importance of this observation is also evident from  
177 the fact that the MIC of amphotericin B was decreased from 1.95 µg/mL to 0.97  
178 µg/mL when the drug was encapsulated in *O*-heptyl nanoparticles.

179         The cytotoxicity of (-)-**S-3d** and amphotericin B when encapsulated in  
180 derivatized nanoparticles was also evaluated using haemolysis and MTT-based  
181 assays (Figure 4). Perhaps unsurprisingly, given that erythrocytes and cell lines  
182 treated with empty dextran nanoparticles (> 90 % cell viability at concentrations of 2  
183 mg/mL) remained completely viable up to 1 mg/mL, the encapsulated, optically  
184 enriched fluconazole analogue (-)-**S-3d** exhibited similar cytotoxicity to that of the  
185 free compound. Thus, essentially no toxicity to two human cell lines (Figures 4b and  
186 4c) was seen when the compound was present at concentrations similar to the MIC  
187 values observed for its anti-fungal activity. The optically enriched fluconazole  
188 analogue (-)-**S-3d** was also considerably less cytotoxic than free amphotericin B in  
189 all assays (Figure 4). It is therefore interesting to note that encapsulating  
190 amphotericin B into *O*-hexadecyl derivatized nanoparticles lowered the cytotoxicity of  
191 this antifungal agent in both the hemolysis and MTT-based assays. Nevertheless,  
192 cell viability was reduced for amphotericin B-containing nanoparticles relative to  
193 derivatized nanoparticles containing fluconazole analogue (-)-**S-3d**.

194 **Conclusions**

195 Reacting alkylamines with a racemic epoxide precursor (Figure 1) in the  
196 presence of immobilized lipase CAL-B in THF provides a simple approach for  
197 the preparation of optically enriched fluconazole analogs, which appear to  
198 exhibit better antifungal activity against *Aspergillus* than fluconazole. Although  
199 the extent to which the enzyme catalyzes the coupling reaction in an  
200 enantioselective manner remains to be determined, we have been able to  
201 assign the (*S*)-configuration to the stereogenic centre of the enantiomer that  
202 exhibits biological activity, assuming that (i) the aminolysis reaction proceeds  
203 with its usual chemical mechanism, and (ii) only one enantiomer has antifungal  
204 activity. Given the difficulty of single-step chemical strategies for the  
205 preparation of chiral fluconazoles in optically enriched form, we anticipate that  
206 the enzymatic methodology reported herein will have significant impact in this  
207 approach to obtaining novel variants of existing antifungal drugs.

208 The most active analogue prepared in this study, (-)-*S*-**3d**, is more  
209 potent against *Aspergillus fumigatus* than fluconazole, having MIC values of 8-  
210 16 µg/mL in a series of *in vitro* assays. Perhaps more importantly for drug  
211 discovery, the anti-*Aspergillus* potency of this compound is enhanced (MIC  
212 1.6-4.0 µg/mL) by encapsulation in derivatized nanoparticles, with minimal *in*  
213 *vitro* cytotoxic effects at concentrations of up to 2 mg/mL against human  
214 erythrocytes and cell lines of human origin.

215

216 **Materials and Methods**

217 ***General procedure for the CAL-B catalysed synthesis of optically***  
218 ***enriched fluconazole analogues.*** CAL-B immobilized on accurel beads (300

219 mg) was added to a solution of the epoxide ( $\pm$ )-**1** (5.0 mmol) and the appropriate  
220 amine (**2a-j**, **4a** or **4b**, 2.5 mmol) dissolved in THF, and the mixture incubated at 55  
221 °C. The extent of the reaction was monitored by TLC and the enzyme was removed  
222 by filtration when the amine was consumed. After removal of THF at reduced  
223 pressure, the residue was subjected to column chromatography using chloroform/  
224 methanol as eluent to afford optically enriched samples of pure fluconazole  
225 analogues (-)-**S-3a-3j**, (-)-**S-5a** or (-)-**S-5b** and the unreacted epoxide (+)-**S-1**.

226

227 **(-)-S-2-(2',4'-Difluorophenyl)-1-hexylamino-3-(1'',2'',4'')triazol-1''-yl-prop-**  
228 **an-2-ol (3d)** was obtained as a viscous oil in 80% yield.  $[\alpha]_D^{20}$  -20.3 (c 0.01,  
229 CHCl<sub>3</sub>); IR spectrum (film)  $\mu_{\max}$ : 3315 (OH and NH), 2979, 1620, 1508, 1415, 1267,  
230 1145, 960 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  0.83 (3H, t, J = 7.63 Hz), 1.17-1.33  
231 (8H, m), 2.43 (2H, t, J = 6.87 Hz), 2.81 (1H, d, J = 12.97 Hz), 3.12 (1H, d, J = 12.21  
232 Hz), 4.49 (1H, d, J = 14.50 Hz), 4.58 (1H, d, J = 13.73 Hz), 6.74-6.82 (2H, m), 7.50-  
233 7.55 (1H, m), 7.77 (1H, s) and 8.10 (1H, s). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  13.92,  
234 22.46, 26.55, 29.83, 31.49, 50.00, 54.14 (d, J<sub>CF</sub> = 3.83 Hz), 55.98 (d, J<sub>CF</sub> = 4.79 Hz),  
235 72.96 (d, J<sub>CF</sub> = 5.75 Hz), 104.12 (d, J<sub>CF</sub> = 26.84 Hz), 111.38 (d, J<sub>CF</sub> = 20.61 Hz), 125.05  
236 (d, J<sub>CF</sub> = 13.42 Hz), 129.79 (d, J<sub>CF</sub> = 6.71 Hz), 144.60, 151.09, 158.92 (d, J<sub>CF</sub> =  
237 237.78 Hz) and 162.29 (d, J<sub>CF</sub> = 249.20 Hz). HRMS: m/z 339.1991 ([M+H]<sup>+</sup>,  
238 C<sub>17</sub>H<sub>25</sub>F<sub>2</sub>N<sub>4</sub>O calcd. 339.1969).

239

240 **Microbroth dilution assay** Various concentrations of different derivatives in the  
241 range of 0.24-1000.0  $\mu$ g/mL were prepared in 96 well culture plates (Nunc, Roskilde,  
242 Denmark) by serial dilution in Sabouraud dextrose broth. Wells were inoculated with  
243  $1 \times 10^6$  spores (conidia) of *Aspergillus* in 10  $\mu$ L of spore suspension. Negative

244 controls were solvent in medium and spores only, with amphotericin B and  
245 fluconazole being used as positive controls. Plates were incubated at 37 °C using a  
246 BOD incubator (Calton, NSW, India) and examined macroscopically after 48 h for the  
247 growth of *Aspergillus* mycelia. The activity of the analogues was defined as positive  
248 if the medium appeared clear without any growth of *Aspergillus* mycelia, and the  
249 minimum concentration of compounds inhibiting growth was reported as MIC (Table  
250 1).

251

252 **Disc diffusion assay** Autoclaved Sabouraud dextrose agar (SDA) was poured into  
253 radiation-sterilized petri dishes (10.0 cm diameter). A suspension of conidia of  
254 *Aspergillus* was prepared and overlaid on the agar plates. Different concentrations of  
255 the fluconazole analogues were impregnated on 5.0 mm diameter sterilized discs  
256 (Whatman No. 1) and placed on the agar. Control discs containing solvent,  
257 amphotericin B or fluconazole were also included in the assay. Plates were  
258 incubated at 37 °C and the zone of inhibition determined after 72 h. MICs reported  
259 for this assay (Table 1) correspond to fluconazole analogue concentrations giving a  
260 zone of inhibition of at least 6.0 mm diameter from the centre of the plate.

261

262 **Percent spore germination inhibition assay** Serial dilutions, ranging from 0.24-  
263 1000.0 µg/mL, of each fluconazole analogue dissolved in Sabouraud dextrose broth  
264 were placed in radiation-sterilized petri dishes (10.0 cm diameter), with each dish  
265 then being inoculated with  $100 \pm 5$  *Aspergillus* conidia. After incubation for 16 h at 37  
266 °C, wells were examined for spore germination using an inverted microscope (Nikon  
267 Diphot, Japan), and the number of germinated, and non-germinated, spores

268 recorded. MICs in this assay (Table 1) correspond to fluconazole analog  
269 concentrations resulting in inhibition of spore germination.

270

271 ***In vitro cytotoxicity assays.*** Two approaches were performed to assess the  
272 cytotoxicity of the fluconazole analogues. First, using a standard haemolytic assay  
273 [28], erythrocytes from healthy individuals were suspended in phosphate buffered  
274 saline (PBS) to give a 2 % suspension (v/v). These cells were then incubated with  
275 various concentrations of each compound for 1 h at 37 °C before being pelleted by  
276 centrifugation at 3000 x *g* for 10 min. The percentage haemolysis was then  
277 calculated from the optical density at 450 nm of the supernatant (Figure 4a). The  
278 effect of solvent and PBS on erythrocyte viability was also checked. Triton X-100  
279 (Sigma Chemicals, USA) was used for complete haemolysis of the erythrocytes.

280 In an alternate approach, an MTT-based assay [29] was used to examine the  
281 cytotoxicity of the analogues against A549 (human pulmonary epithelial cells)  
282 and U87 (primary glioblastoma cells) human cell lines, obtained from National  
283 Centre for Cell Science, Pune, India (Figures 4b and 4c). Briefly, cells were  
284 cultured in RPMI-1640 medium supplemented with L-glutamine and fetal calf  
285 serum (10 % v/v), before being harvested at the log phase of confluency and  
286 re-suspended in RPMI-1640 medium. Samples ( $2 \times 10^4$  cells in 100  $\mu$ L) were  
287 seeded into culture plates and allowed to grow overnight at 37 °C under 5 %  
288 (v/v) CO<sub>2</sub>. Fluconazole analogues were added at a variety of concentrations  
289 and the cells were incubated under the same conditions for 24 h. Equivalent  
290 amounts of solvent, amphotericin B and fluconazole were used as negative  
291 and positive controls. The medium was removed from each well before the  
292 addition of 50.0  $\mu$ g of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

293 bromide (MTT) in PBS (100  $\mu$ L). After incubation for a further period of 4 h at  
294 37 °C, the MTT solution was removed and the cells were lysed using  
295 isopropanol-HCl (100.0  $\mu$ L). The absorption of each well (at 540 nm) was used  
296 to determine the percentage cytotoxicity in a micro-plate reader (Spectra max  
297 384 plus, Molecular Devices, USA).

298

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305

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401 **Figure Captions**

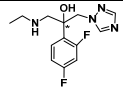
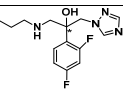
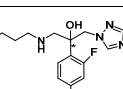
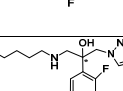
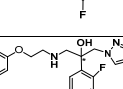
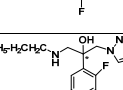
402 **Figure 1.** CAL-B catalyzed epoxide ring opening with open chain and cyclic aliphatic  
403 amines. Note that samples of each compound could also be prepared in racemic  
404 form by heating the epoxide and amine at 55 °C in THF (see Supporting  
405 Information). The new stereogenic centre is indicated by an asterisk.

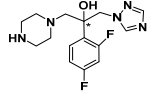
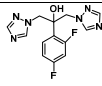
406 **Figure 2.** *In vitro* release of (-)-**S-3d** from *O*-hexadecyl- (blue triangles), *O*-decyl-  
407 (red circles) and *O*-heptyl-derivatized (grey squares) dextran nanoparticles.

408 **Figure 3.** *In vitro* antifungal activity of (-)-**S-3d**, amphotericin B and their  
409 dextran NPs. Lane a: Negative control; Lane b: Empty *O*-alkyl dextran  
410 nanoparticles; Lane c: Amphotericin B; Lane d: Fluconazole; Lane e: (-)-**S-3d**;  
411 Lane f: *O*-heptyl nanoparticles containing (-)-**S-3d**; Lane g: *O*-decyl  
412 nanoparticles containing (-)-**S-3d**; Lane h: *O*-hexadecyl nanoparticles  
413 containing (-)-**S-3d**; Lane i: *O*-heptyl nanoparticles containing Amphotericin B;  
414 Lane j: *O*-decyl nanoparticles containing Amphotericin B; Lane k: *O*-hexadecyl  
415 nanoparticles containing Amphotericin B.

416 **Figure 4.** *In vitro* cytotoxicity assays for optically enriched (-)-**S-3d** and amphotericin  
417 B in both the free form and when encapsulated into dextran nanoparticles. (a)  
418 Haemolytic assay; MTT-based assay using (b) A459 and (c) U87 cell lines.

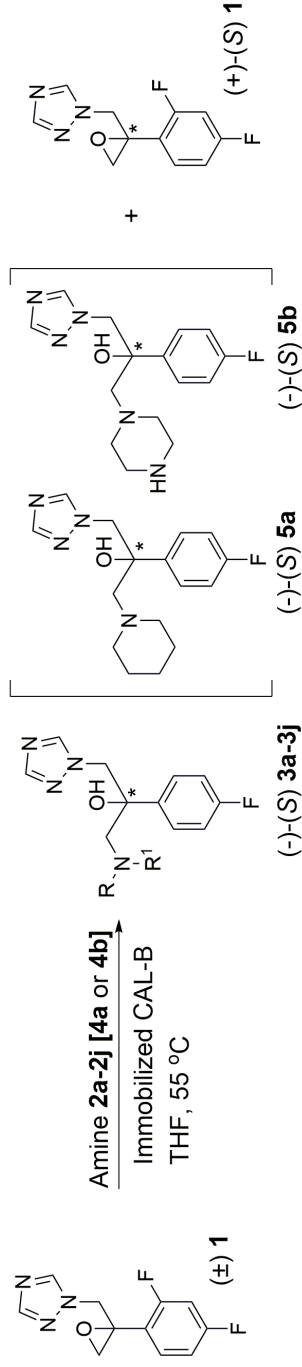
**Table 1:** *In vitro* activity of selected, optically enriched fluconazole analogues against *Aspergillus* species.<sup>a,b</sup>

	Analogue	<i>Aspergillus fumigatus</i>			<i>Aspergillus niger</i>			<i>Aspergillus flavus</i>		
		MDA ( $\mu\text{g/ml}$ )	DDA ( $\mu\text{g/disc}$ )	PSGI ( $\mu\text{g/ml}$ )	MDA ( $\mu\text{g/ml}$ )	DDA ( $\mu\text{g/disc}$ )	PSGI ( $\mu\text{g/ml}$ )	MDA ( $\mu\text{g/ml}$ )	DDA ( $\mu\text{g/disc}$ )	PSGI ( $\mu\text{g/ml}$ )
(-)-S-3a		125.0 (250.0)	62.50 (125.0)	125.0 (250.0)	250.0 (250.0)	125.0 (125.0)	250.0 (250.0)	250.0 -	125.0 -	250.0 -
(-)-S-3b		250.0 (250.0)	62.50 (125.0)	250.0 (250.0)	500.0 -	125.0 -	500.0 -	- -	- -	- -
(-)-S-3c		62.50 (125.0)	31.25 (31.25)	62.50 (125.0)	62.50 (500.0)	31.25 (125.0)	62.50 (500.0)	500.0 (1000.0)	125.0 (125.0)	500.0 (1000.0)
(-)-S-3d		15.62 (15.62)	7.81 (7.81)	15.62 (15.62)	62.50 (125.0)	15.62 (31.25)	62.50 (125.0)	125.0 (125.0)	15.62 (31.25)	125.0 (125.0)
(-)-S-3e		62.50 (125.0)	31.25 (62.50)	62.50 (125.0)	125.0 (500.0)	62.50 (125.0)	125.0 (500.0)	500.0 (500.0)	125.0 -	500.0 (500.0)
(-)-S-3f		500.0 (500.0)	250.0 (250.0)	500.0 (500.0)	500.0 -	125.0 -	500.0 -	500.0 -	250.0 -	500.0 -

(-)- <b>S-5b</b>		62.50 (125.0)	31.25 (62.50)	62.50 (125.0)	125.0 (500.0)	62.50 (125.0)	125.0 (500.0)	500.0 (500.0)	125.0 (125.0)	500.0 (500.0)
	 Fluconazole	250.0	125.0	250.0	250.0	125.0	250.0	250.0	125.0	250.0
	Amphotericin B	1.95	0.97	1.95	1.95	0.97	1.95	1.95	0.97	1.95

<sup>a</sup>Values in parentheses are for the racemic form of the compound.

<sup>b</sup>(-) shows no activity within the range of concentrations tested.



- 2a** or **3a**: R = H, R<sup>1</sup> = CH<sub>2</sub>CH<sub>3</sub>  
**2b** or **3b**: R = H, R<sup>1</sup> = CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>  
**2c** or **3c**: R = H, R<sup>1</sup> = CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>  
**2e** or **3e**: R = H, R<sup>1</sup> = CH<sub>2</sub>CH<sub>2</sub>OPh  
**2f** or **3f**: R = CH<sub>3</sub>, R<sup>1</sup> = CH<sub>2</sub>(CH<sub>2</sub>)<sub>16</sub>CH<sub>3</sub>  
**2g** or **3g**: R = H, R<sup>1</sup> = CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub>  
**2h** or **3h**: R = H, R<sup>1</sup> = CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>  
**2i** or **3i**: R = H, R<sup>1</sup> = CH<sub>2</sub>CH<sub>2</sub>Ph  
**2j** or **3j**: R = H, R<sup>1</sup> = CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>Ph

