The in vitro Antimycotic Activity and Acute Toxicity of Third-Generation Benzylidenetetralones and Heteroarylidenetetralones

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Key Words

Benzylidenetetralones · Heteroarylidenetetralones · Antimycotic activity, in vitro · Acute toxicity, in vivo/in vitro

Abstract

Objectives: A new series of E-2-arylmethylene-1-tetralones and E-2-heteroarylmethylene-1-tetralones (third generation) were designed as potential antimycotic agents against human pathogenic yeasts. **Methods:** The new compounds were obtained by aldol condensation of 1-tetralone with the appropriately substituted aldehydes in either acid- or base-catalysed conditions. These compounds were tested for their in vitro antimycotic activity against 24 strains of *Cryptococcus neoformans*, *Candida* spp. and *Trichosporon cutaneum* by a microtitre well technique in a liquid casitone medium, using a double dilution method. The toxicity of the new compounds was determined in vitro in cultured HeLa cells, in HeLa growth medium and in vivo in MFI mice. **Results:** Nineteen new E-2-benzylidene-1-tetralones were prepared, and 16 of the tested compounds showed superior antimycotic activity when compared to the first-generation

E-2-benzylidene-1-tetralones synthesised previously. Twelve of these third-generation compounds were more active against different strains of yeasts than 6 commercial antimycotic agents similarly tested. An in vitro toxicity study and an in vivo acute toxicity study in MFI mice showed that these compounds were severalfold less toxic than most of the commercial antimycotics, with 4 being comparable to or less toxic than the least toxic of the commercial agents (amphotericin B) tested by the same procedures. **Conclusions:** Twelve members of this new E-2-benzylidene-1-tetralone class of compounds are promising candidates for antimycotic agents worthy of further investigation.

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Introduction

We have previously reported the aldol condensation of 1-tetralones with appropriately substituted aldehydes in the presence of weak bases such as piperidine or pyrrolidine, which were shown to yield E-2-benzylidene derivatives at room temperature [1–5]. Although these first-generation E-2-benzylidene-1-tetralones were shown to ex-

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Fig. 1. Structure of the 19 new compounds, with aryl or heteroaryl groups.

Table 1. Substitution pattern of the 19 E-2-benzylidene-1-tetralone analogues synthesised

Compound	Aryl	R ₁	R ₂	R ₃	R ₄
1	–	H	OCH ₂ Ph	H	H
2	–	H	H	COOH	H
3	–	H	OSO ₂ CH ₃	H	H
4	–	SO ₃ H	H	H	H
5	–	OCH ₃	H	H	H
6	–	H	O(CH ₂) ₃ CH ₃	H	H
7	–	H	OH	H	H
8	–	Cl	H	H	H
9	–	H	Cl	Cl	H
10	–	Cl	H	Cl	H
11	–	Cl	H	H	Cl
12	–	H	H	CN	H
13	2'-(1'-N-methyl)-pyrrolyl				
14	2'-pyridyl				
15	2'-thienyl				
16	3'-pyridyl				
17	4'-pyridyl				
18	2'-furyl				
19	2'-pyrrolyl				

hibit very low toxicity in vivo [3], their in vitro activity against some pathogenic yeasts was found to be discouraging [2–4]. When the new aldol condensation, performed in ethanolic sodium hydroxide at 0°C, was utilised to synthesise another generation of E-2-benzylidene- or E-2-het-

eroarylidene-1-tetralones (compounds 1–12 or 13–19, respectively), the condensation products obtained also had the E configuration (fig. 1). Carrying out the condensation with prolonged heating under acidic conditions with orthophosphoric acid (at 80°C) also exclusively yielded the E-2-arylidene derivatives (fig. 1). Using these two newer reaction conditions, 19 new compounds were synthesised having a variety of aryl or heteroaryl groups (fig. 1; table 1).

The antimycotic activity of these new compounds was assessed in vitro using a microtitre technique, against 24 strains of *Cryptococcus neoformans*, *Candida* spp. and *Trichosporon cutaneum*, and compared to that of 6 commercially available antimycotic agents tested simultaneously [2–6].

The toxicities of the compounds prepared were investigated both in vitro in a HeLa cell line [7, 8] as well as by an acute toxicity procedure in MFI mice [3, 7–9], and the results were compared with those of 6 commercial antimycotic agents, tested similarly.

Materials and Methods

A general base-catalysed method for the synthesis of the E-2-arylidene-1-tetralones (1–19) was used. 1-Tetralone (0.01 mol) and the corresponding aldehyde (0.01 mol) were dissolved in ethanol (150 ml), and sodium hydroxide solution (30 ml, 6.7 M) was added to the reaction mixture at 4°C. The separated crystals were filtered, washed with cold ethanol and distilled water until the pH of the wash-out was neutral.

A general acid-catalysed method for the synthesis of the E-2-arylidene-1-tetralones (1–19) was also used. 1-Tetralone (0.01 ml) and the appropriate aldehyde (0.01 mol) were dissolved in phosphoric acid (85%, 25 ml). The reaction mixture was stirred under argon atmosphere in an oil bath at 80°C for 6 h, cooled to ambient temperature and diluted with water. The oily solution was extracted several times with chloroform (3 × 100 ml), and the combined chloroform extracts were washed with water until the wash-out became neutral. The extract was dried on anhydrous magnesium sulphate. The products obtained by both the base- and acid-catalysed procedures were found to be identical by chemical characterisation as described below.

The newly synthesised compounds 1–19 were obtained in moderate to high yields (21–99%). The products were recrystallised from methanol. Some of them required further purification by silica gel column chromatography using the eluents benzene for 1, benzene-acetone (40:1 v/v) for 3 and benzene-hexane (9:1 v/v) for 6. Their physical data, listed in table 2, were in accordance with the analogous compounds published elsewhere [10] or – for compounds 13, 15 and 18 – were found to be identical with those reported previously [11]. The pure compounds were characterised by NMR and infrared spectroscopic methods and elemental analysis. In particular, the appearance of the H-α proton signal in the range of δ 8.50–7.60 ppm in the ¹H-NMR spectra unequivocally corroborated their E configuration [12, 13].

For in vitro antimycotic activity, the compounds were dissolved in DMSO (analar), and the activity was tested by a microtitre well technique in triplicate sets, in a liquid casitone medium. A double dilution method within the concentration range of 0.8–100 µg/ml was used against 24 strains of *C. neoformans*, *Candida* spp. and *T. cutaneum*, employing the procedure reported earlier [2–4, 7]. The cells which were originally hospital isolates from different hospitals in Kuwait and Ireland were maintained by routine subculture on subaroid solid media and were classified using conventional methods [2, 4, 14]. The level of DMSO in any well did not exceed 2.5% (v/v), which was found to be non-inhibitory to fungal growth in control wells [2–4, 7]. For assessment of the level of the antimycotic activity of the compounds prepared in this investigation, 6 commercial antimycotic agents were also tested simultaneously using the same experimental conditions. The commercial agents were: miconazole, isconazole, sertaconazole, amphotericin B, haloprogin and tolnaftate. Compounds exhibiting minimum inhibitory concentration (MIC) values of 6 µg/ml or lower were considered to display good antimycotic activity.

The toxicities of the new compounds and the references compounds listed above were determined in vitro in cultured HeLa cells (Ohio) and in a HeLa growth medium [14, 15] in triplicate sets at each concentration over a concentration range of 1–500 µg/ml per culture bottle. The cells in each culture bottle were harvested and counted using a haemocytometer. The survival of the cells in the culture bottles was expressed as a percentage of the control culture cells (without any added compound or agent). A two-tailed Anova model in SPSS for Windows (version 6.1.1) was used for statistical analysis. The values of the inhibitory concentrations (IC₅₀; µg/ml) were then determined from the graph for each compound or agent, as described earlier by us [6, 7].

The in vivo toxicity of the compounds 1–19 compared to the 6 commercial agents was assessed in triplicate sets of 10 MFI mice using an acute toxicity procedure [9, 15]. The LD₅₀ values were considered valid for any group of mice, per compound or agent, when the survival coefficient of variance for the particular dose used was within 3–5% [6, 9].

Results

A list of the 19 compounds synthesised and the substitution pattern are shown in table 1. Their physical data are listed in table 2. The MIC values for active compounds (6 µg/ml or lower) as a measure of their in vitro antimycotic activity, and those of the 6 commercial antimycotic agents (12.5 µg/ml or lower) against the 24 strains of yeasts tested, are shown in table 3.

The inhibitory concentrations for each compound or agent determined by in vitro toxicity testing in HeLa cell lines and calculated from the mean survival counts' curves for each compound or agent using the SPSS package are listed in table 4. The LD₅₀ values for compounds 1–19 and the 6 commercial agents (mg/kg weight) provide a measure of their in vivo toxicity in MFI mice (table 4).

Table 2. Physical data for compounds 1–19

Compound	Melting point °C	Yield %	Molecular formula
1	45–46	80 ¹	C ₂₄ H ₂₀ O ₂
2	240–242	99	C ₁₈ H ₁₄ O ₂
3	95–97	22 ²	C ₁₈ H ₁₆ O ₄ S
4	149 (dec.)	21	C ₁₇ H ₁₄ O ₃ S
5	109–111	80	C ₁₈ H ₁₆ O ₂
6	– ³	37 ⁴	C ₂₁ H ₂₂ O ₂
7	152–153	40	C ₁₇ H ₁₄ O ₂
8	73–74	86	C ₁₇ H ₁₃ ClO
9	152–154	63	C ₁₇ H ₁₂ Cl ₂ O
10	108–109	90	C ₁₇ H ₁₂ Cl ₂ O
11	66–67	40	C ₁₇ H ₁₂ Cl ₂ O
12	162–163	77	C ₁₈ H ₁₃ ON
14	122–123	30	C ₁₆ H ₁₃ NO
16	75–76	32	C ₁₆ H ₁₃ NO
17	114–115	59	C ₁₆ H ₁₃ NO
19	180–182	63	C ₁₅ H ₁₃ NO

dec. = Decomposition.

¹ 19% of starting material was recovered.

² 71% of starting material was recovered.

³ Oily compound.

⁴ 40% of starting material was recovered.

Discussion

Nineteen members of this third generation of E-2-benzylidene-1-tetralones (table 1) were synthesised under the new strong acid and base reaction conditions as we intended to introduce heteroaromatic compounds (13–19), polychlorinated (9–11), ionisable hydroxyl sulphonic and acidic groups [2, 4, 7] as well as new ether, ester (1, 3, 5, 6) and cyano (12) residues as substituents for comparison to the first and second generation 1–4 and 14 E-2-benzylidene-1-tetralones, respectively. The broad spectrum of antimycotic activity exhibited by 10 of the new compounds (table 3) was generally dependent on the presence of free or ionisable acidic-oxygenated functional groups (2, 4, 7) or nitrogenous residues having free or non-methylated nitrogen atoms, and specifically when the nitrogen substituent was a 6-membered ring (pyridyl derivatives; 14, 16, 17) and, to a lesser extent, when the nitrogen residue was that of a 5-membered pyrrole ring (19).

Masking of either the free oxygen or the nitrogen atoms (1, 6, 13) resulted in the loss of activity, except in the case of the mesyloxy compound (3), which was shown to be

Table 3. The MIC ($\mu\text{g/ml}$) values of the active E-2-benzylidene-1-tetralone analogues and commercial agents, against 24 strains of yeasts

Yeast tested	Active compounds ¹	Commercial agents ²
<i>Cryptococcus neoformans</i>		
sh27/t	2 (3.0); 4 (1.5); 7 (1.5); 11 (6.0); 12 (6.0); 14 (6.0); 15 (6.0); 16 (3.0); 17 (1.5); 18 (6.0)	A (12.5); H (12.5); S (12.5)
ah27/a	2 (3.0); 4 (0.8); 7 (1.5); 11 (6.0); 12 (6.0); 14 (6.0); 15 (6.0); 16 (6.0); 17 (1.5); 18 (6.0)	A (12.5); H (12.5); T (12.5); S (12.5)
ahh1/j	2 (3.0); 4 (1.5); 7 (1.5); 14 (6.0); 15 (6.0); 16 (6.0); 17 (1.5)	A (6); H (12.5); T (12.5); S (12.5)
ahh9/r	2 (3.0); 4 (1.5); 7 (1.5); 11 (6.0); 14 (6.0); 15 (6.0); 16 (6.0); 17 (3.0); 18 (6.0)	A (12.5); H (12.5); S (6.0)
mk53/k	2 (6.0); 4 (1.5); 7 (3.0); 12 (6.0); 16 (3.0); 17 (3.0)	A (12.5); H (12.5); S (6.0)
ahh2/n	2 (6.0); 4 (1.5); 7 (1.5); 12 (6.0); 16 (3.0); 17 (1.5)	H (6); S (12.5)
<i>Candida albicans</i>		
am/t27	3 (6.0); 7 (6.0); 11 (6.0)	A (12.5); H (12.5); S (12.5)
sh/t54	3 (6.0); 7 (6.0); 11 (6.0); 14 (6.0); 17 (6.0)	A (12.5); H (12.5); S (12.5)
mk/b65	3 (6.0); 7 (6.0); 17 (6.0)	H (12.5); S (6.0)
jjg/j68	–	S (6.0)
tc/d92	3 (6.0); 7 (6.0); 11 (6.0)	A (12.5); H (12.5); S (12.5)
lm/s93	3 (6.0); 11 (6.0)	H (12.5); S (12.5)
<i>Candida parapsilosis</i>		
ah/t42	3 (6.0); 7 (6.0); 10 (6.0); 14 (6.0); 18 (6.0)	A (12.5); H (6.0); T (12.5); S (12.5)
mk/j68	3 (6.0); 9 (6.0); 19 (6.0); 18 (6.0)	H (12.5); T (12.5); S (6.0)
jjg/j69	3 (6.0); 9 (6.0); 14 (6.0); 18 (6.0)	A (12.5); H (12.5); S (6.0)
<i>Candida tropicalis</i>		
cp/k92	11 (6.0); 17 (3.0); 18 (3.0)	A (12.5); H (12.5); T (12.5); S (6.0)
cp/k91	11 (6.0); 17 (3.0); 18 (1.5)	A (6); H (12.5); S (12.5)
cp/v91	11 (6.0); 17 (3.0); 18 (1.5)	A (12.5); H (6); S (6.0)
<i>Candida glabrata</i>		
t/92k	3 (6.0); 9 (6.0); 14 (6.0); 15 (3.0); 19 (6.0)	A (6); H (6); S (12.5)
t/91k	3 (3.0); 9 (6.0); 14 (6.0); 15 (3.0); 19 (6.0)	A (12.5); H (12.5); T (6); S (12.5)
k/92j	3 (3.0); 5 (6.0); 14 (6.0); 15 (3.0); 19 (6.0)	A (12.5); H (6); T (12.5); S (6.0)
<i>Trichosporon cutaneum</i>		
cw/92	7 (6.0); 9 (3.0); 12 (3.0); 14 (6.0); 15 (6.0); 17 (3.0)	A (6.0); H (12.5); T (12.5); S (6.0)
jk/92	7 (6.0); 9 (1.5); 12 (3.0); 14 (3.0); 15 (6.0); 17 (3.0)	A (12.5); H (6); T (12.5); S (6.0)
vx/91	7 (6.0); 9 (3.0); 12 (3.0); 14 (3.0); 15 (6.0); 17 (6.0)	A (12.5); H (12.5); T (12.5); S (12.5)

MIC values determined in vitro in liquid casitone medium. A = Amphotericin B; H = haloprogin; T = tolinaftate; S = sertaconazole.

¹ Compound numbers are accompanied by their MIC values ($\mu\text{g/ml}$) in parentheses. Compounds with a MIC of 6 $\mu\text{g/ml}$ or lower are considered to reflect good antimycotic activity.

² MIC values greater than 12.5 $\mu\text{g/ml}$ for commercial agents are not quoted, those of 12.5 $\mu\text{g/ml}$ or lower are shown in parentheses.

active against *C. albicans*, *C. parapsilosis* and *C. glabrata* strains only. The most active commercial agents tested were sertaconazole and amphotericin B, exhibiting good activity in the majority of the strains (MIC values of 12.5 or 6 $\mu\text{g/ml}$). None of the compounds was active against the *C. albicans* strain (jjg/j68), where only sertaconazole of the commercial agents exhibited good activity.

Although the simple E-2-benzylidene-1-tetralones (1–12) were overall less toxic than those where the phenyl group was replaced with a heterocyclic moiety (13–19), as

reflected by their LD₅₀ values in mice, many were comparably or slightly more toxic than the least toxic commercial agent, amphotericin B. When compared to the most active of the commercial agents, sertaconazole, the in vivo toxicity of the new compounds was found to be marginally better. In particular, those compounds with carboxyl (2) and mesyloxy substituents (3) were about 11 and 6% less toxic than sertaconazole. The toxicity differences between those for amphotericin B and sertaconazole relative to the compounds 1–19 were more amplified in cell

Table 4. In vivo and in vitro toxicity for the 19 new E-2-benzylidene-1-tetralone analogues and the 6 commercial antimycotic agents tested

	In vivo LD ₅₀ mg/kg	In vitro IC ₅₀ µg/ml	p value
<i>Compound</i>			
1	>350	291.4	0.001
2	256	199.5	0.001
3	242	215.5	0.001
4	165	106.9	0.001
5	179	90.4	0.012
6	>350	263.1	0.001
7	183	82.2	0.008
8	236	120.1	0.030
9	212	153.2	0.001
10	209	147.2	0.001
11	216	156.5	0.041
12	198	122.3	0.001
13	152	81.4	0.038
14	163	84.4	0.007
15	138	61.4	0.008
16	145	72.8	0.022
17	131	52.9	0.046
18	103	30.9	0.048
19	129	43.6	0.008
<i>Commercial agent</i>			
M	220	18.8	0.002
I	189	21.8	0.018
H	89	5.2	0.005
T	120	7.6	0.016
A	260	36.4	0.014
S	228	14.9	0.008

p values were obtained from mean cell counts at different concentrations for each compound or commercial agent in test culture flasks, as a percentage of control culture flasks where neither compounds nor agents were added. M = Miconazole; I = isoconazole; H = haloprogin; T = tolinaftate; A = amphotericin B; S = sertaconazole.

culture, where all 10 active compounds (2, 4, 7, 9, 11, 12, 14, 16, 17, 19) were found to be 5- to 15-fold less toxic than either of the 2 commercial agents described above.

The 2 most non-toxic compounds, either in vitro or in vivo, in the series were compounds 1 and 6. Unfortunately these 2 compounds exhibited weak antimycotic activity against the strains utilised in this study.

The mechanism of action of other α,β -unsaturated ketones has been studied by Dimmock and Wong [16], and it is suggested that it is due to the alkylation of the thiol enzymes. Although the mechanism of action of these third-generation compounds has not been studied, we speculate that their activity would decrease in the presence of such thiols as thioglycolic acid, cysteine and glutathione.

Conclusion

We have synthesised 19 E-2-arylidene-1-teralones that displayed in vitro antimycotic activity against 24 miconazole-resistant fungi. The compounds of highest activity contain ionisable oxygenated functional groups or basic groups with unsubstituted cyclic nitrogen atoms.

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