

# *In Vitro* Antifungal Activity of 2-(4-Substituted Phenyl)-3(2*H*)-Isothiazolones

N. ADIBPOUR<sup>a</sup>, A. KHALAJ<sup>b</sup>, S. REZAEI<sup>c</sup>, M. DANESHTALAB<sup>d</sup>

<sup>a</sup>Department of Medicinal Chemistry, Ahvaz Medical Sciences University, P.O. Box 61357-33184, Ahvaz, Iran

fax +98 611 334 0988

e-mail n.adibpour@ajums.ac.ir

<sup>b</sup>Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran Medical Sciences University, PO Box 14155/6451, Tehran, Iran

<sup>c</sup>Department of Pharmaceutics, Ahvaz Medical Sciences University, PO Box 61357-33184, Ahvaz, Iran

<sup>d</sup>School of Pharmacy, Health Sciences Centre, Memorial University of Newfoundland, St. John's (NL), A1B 3V6, Canada

Received 24 April 2006

Revised version 30 May 2007

**ABSTRACT.** The *in vitro* antifungal activity of several N<sup>2</sup>-phenyl-3(2*H*)-isothiazolones substituted at C<sup>4</sup> of the phenyl moiety with heterocyclic nucleus or groups of different physico-chemical properties against four human pathogenic fungi was determined by broth macrodilution method; results were compared with those obtained with itraconazole and ketoconazole. These isothiazolones showed moderate to high activity against some or all tested strains and in comparison with the reference drugs, 5-chloro-2-(4-nitrophenyl)isothiazol-3-one (**1g**), 5-chloro-2-phenylisothiazol-3-one (**1c**), 4-[4-(5-chloro-3-oxo-3*H*-isothiazol-2-yl)phenyl]-1,4-dihydrotriazol-5-one (**1s**) and 2-(4-nitrophenyl)isothiazol-3-one (**2g**) against *Aspergillus niger*, 5-chloro-2-(4-nitrophenyl)isothiazol-3-one (**1g**) and 4-[4-(5-chloro-3-oxo-3*H*-isothiazol-2-yl)phenyl]piperazine-1-carboxamide (**1q**) against *Trichophyton mentagrophytes* had comparable activity, compounds **1g** and **2g** showing higher activity against *Microsporum canis*. Antifungal activity was favored by the presence of chlorine at C<sup>5</sup> of the isothiazolone and/or the presence of nitro group or heterocyclic nucleus at C<sup>4</sup> of the phenyl ring and proper hydrophilicity of the molecule.

## Abbreviations

MIC(s) minimal inhibitory concentration(s)

Mops 3-morpholinopropanesulfonic acid

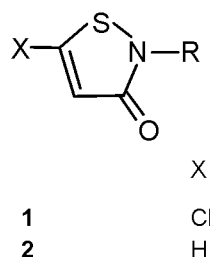
Isothiazol-3(2*H*)-ones substituted at N<sup>2</sup> with alkyl and aryl derivatives (Lewis *et al.* 1973), active halogen compounds (Lewis and Miller 1974), certain acyl derivatives (Lewis and Miller 1970), carbamoyl groups (Lewis *et al.* 1969), organotin derivatives (Lewis and Miller 1972) with or without substitutions at C<sup>4</sup> and/or C<sup>5</sup> have shown varying biocidal activity (Lewis *et al.* 1978) and have been subject of numerous patents for a wide range of industrial applications to control living organisms. These compounds are resistant to inhibition by common additives and as such have been used as preservatives in foods and cosmetics (Lewis *et al.* 1981).

Khalaj *et al.* (2004) described the synthesis and evaluation of *in vitro* antibacterial activity of several novel and known N<sup>2</sup>-phenyl-3(2*H*)-isothiazolones substituted at the C<sup>4</sup> of the phenyl ring with groups different in hydrophobicity, size, steric, and electronic properties. Most of compounds exhibited moderate to high activity against certain G<sup>+</sup>- and G<sup>-</sup>-bacteria and in comparison with ciprofloxacin, ceftriaxon, ceftazidim, and gentamicin (as reference drugs), some compounds showed comparable or higher activity. Among the examined compounds were those bearing piperazine (**1o–r**) or 2*H*-1,2,4-triazol-3(4*H*)-one (**1s**) rings which are present in some azole antifungals and have been reported to improve both pharmacokinetic and pharmacodynamic behavior (Ji *et al.* 2000; cf. Jantová *et al.* 2005).

While the antifungal activity of **1b** and **2c,e** (Lewis *et al.* 1981) against *A. niger* by serial dilution method has been described, no report on the activity of these and other tested compounds against pathogenic fungi by any other method is available. Therefore a comparative antifungal study to determine the effect of substituents, especially heterocyclic derivatives, on the activity appeared of interest. This paper describes the *in vitro* antifungal activity of compounds **1** and **2** against clinical isolates of *A. niger*, *C. albicans*, *T. mentagrophytes* and *Microsporum canis* in comparison with itraconazole and ketoconazole by the broth macrodilution method (Messick *et al.* 1999).

## MATERIALS AND METHODS

*Chemical compounds.* For synthesis of compounds **1** and **2** through addition of CH<sub>2</sub>Cl<sub>2</sub> solution of SO<sub>2</sub>Cl<sub>2</sub> (Merck, Germany) as an oxidizing agent to the solution of dithiodipropionamides in the same solvent at 0–10 °C see Khalaj *et al.* (2004).



X

**1**

Cl

**2**

H

<b>1a</b>	R H	<b>1c, 2c</b>	R' H
<b>1b</b>		<b>1d, 2d</b>	Me
<b>1c-s, 2c-n</b>		<b>1e, 2e</b>	Cl
		<b>2f</b>	SMe
		<b>1g, 2g</b>	NO <sub>2</sub>
		<b>1h, 2h</b>	CF <sub>3</sub>
		<b>1i, 2i</b>	OMe
		<b>1j, 2j</b>	OEt
		<b>1k, 2k</b>	OPr
		<b>1l</b>	OAc
		<b>1m, 2m</b>	COOEt
		<b>1n, 2n</b>	NMe <sub>2</sub>
<b>1o</b>	R'' H	<b>1o-r</b>	
<b>1p</b>			
<b>1q</b>	-CONH <sub>2</sub>	<b>1s</b>	
<b>1r</b>	-COOEt		

*Antifungal activity.* The *in vitro* activity against clinical isolates of *A. niger*, *C. albicans*, *T. mentagrophytes* and *M. canis* were evaluated in the Department of Parasitology and Mycology, School of Public Health, Tehran Medical Sciences University. Prior to testing each isolate was subcultured on Sabouraud dextrose agar (Difco, UK) at 37 °C to ensure optimum growth characteristics and purity. The incubation times were 1–2 d for *C. albicans*, 2–3 d for *A. niger*, and 5 d for *T. mentagrophytes* and *M. canis*. For dermatophyte suspensions of each strain in 3 mL distilled water containing 500 ppm (V/V) Tween 80 (Sigma, USA) were prepared by agitation with a vortex shaker. After removal of the hyphal fragments and agar blocks by filtration through a sterile celite, the starting inocula were adjusted to 10<sup>6</sup> CFU/mL by a spectrophotometer set at 530 nm and diluted with distilled water to a final concentration of 10<sup>4</sup> CFU/mL. The yeast cell suspension of *C. albicans* at 10<sup>4</sup> CFU/mL was prepared by similar procedure but without Tween 80.

Stock solutions of the tested compounds, ketoconazole and itraconazole (Sigma, USA) as the reference drugs (1 mg/mL) were prepared in Me<sub>2</sub>SO (1 mL) and solutions were treated on shaking with distilled water (9 mL). The resulting solutions were progressively 2× diluted with the test medium which was RPMI 1640 (Sigma, USA) supplemented with L-glutamine without sodium hydrogencarbonate and buffered to pH 7 with 0.165 mol/L (35.5 g/L) Mops to give the final range of 0.01–100 µg/mL. Blanks were prepared in the test medium with the same quantity of H<sub>2</sub>O and Me<sub>2</sub>SO, but without the tested compounds. One mL of each fungus strain was mixed with 1 mL of each media containing the tested compounds or the reference drugs in 5-mL culture tubes; they were incubated at 37 °C for 3 d for *T. mentagrophytes*, and for 2 d for other species. MIC was defined as the lowest concentration of the compound at which there was no

visible growth. Each experiment was repeated in triplicate and mean values were used to compute the MICs (Table I).

**Table I.** *In vitro* antifungal activities of 5-chloro-3(2*H*)-isothiazolones (**1**), 3(2*H*)-isothiazolones (**2**) and standard drugs itraconazole and ketoconazole (MIC,  $\mu\text{g/mL}$ )<sup>a</sup>

Compound	<i>T. men.</i>	<i>M. can.</i>	<i>C. alb.</i>	<i>A. nig.</i>	Compound	<i>T. men.</i>	<i>M. can.</i>	<i>C. alb.</i>	<i>A. nig.</i>
<b>1a</b>	15	25	0.6	25	–				
<b>1b</b>	1.25	10	0.6	25	–				
<b>1c</b>	5	0.3	32	<0.01	<b>2c</b>	15	1.5	30	0.1
<b>1d</b>	5	20	5	50	<b>2d</b>	15	20	1	50
<b>1e</b>	20	1.25	1.25	0.6	<b>2e</b>	25	5	2.5	2.5
–					<b>2f</b>	5	25	12	12.5
<b>1g</b>	<0.01	0.125	0.07	<0.01	<b>2g</b>	0.1	0.1	0.1	<0.01
<b>1h</b>	20	20	>100	25	<b>2h</b>	25	25	>100	100
<b>1i</b>	25	25	50	25	<b>2i</b>	25	25	50	25
<b>1j</b>	5	20	100	50	<b>2j</b>	15	25	100	50
<b>1k</b>	2.5	20	50	>100	<b>2k</b>	5	20	50	50
<b>1l</b>	15	25	100	100	–				
<b>1m</b>	20	5	100	>100	<b>2m</b>	25	20	100	>100
<b>1n</b>	20	20	2.5	25	<b>2n</b>	25	20	12	>100
<b>1o</b>	0.07	5	1.25	0.6	–				
<b>1p</b>	0.15	10	15	50	–				
<b>1q</b>	<0.01	5	32	20	–				
<b>1r</b>	1.25	1.25	0.3	10	–				
<b>1s</b>	5	5	0.15	<0.01	–				
Itraconazole	<0.01	0.25	0.01	<0.01					
Ketoconazole	<0.01	0.25	0.01	<0.01					

<sup>a</sup>*T. men.* – *T. mentagrophytes*

*M. can.* – *M. canis*

*C. alb.* – *C. albicans*

*A. nig.* – *A. niger*

## RESULTS AND DISCUSSION

MIC ranges (<0.01–25  $\mu\text{g/mL}$ ; Table I) for all compounds against *T. mentagrophytes* and *M. canis* were lower (MIC < 0.01–100  $\mu\text{g/mL}$ ) than those against *A. niger* and *C. albicans*.

In contrast to the results of Khalaj *et al.* (2004) where most of the compounds **1** (*i.e.* with Cl at the C<sup>5</sup> of the isothiazolone ring) were less active than the corresponding unsubstituted analogs **2** (with exception of **1c** and **1d** against *C. albicans* and of **1k** against *A. niger*), other Cl<sup>5</sup> derivatives showed higher or comparable antifungal activity in comparison with analogs without Cl<sup>5</sup> substitution. The most potent compound was **1g** which showed equal activity (MIC < 0.01  $\mu\text{g/mL}$ ) against *T. mentagrophytes* and *A. niger* and higher activity against *M. canis* (0.125  $\mu\text{g/mL}$ ) in comparison to itraconazole and ketoconazole. The derivative without Cl<sup>5</sup> (**2g**) was also equally active as the reference drugs against *A. niger* (MIC < 0.01) and showed higher activity (0.1  $\mu\text{g/mL}$ ) against *M. canis*.

The higher antifungal effects of nitrophenyl derivatives **1g** and **2g** which were mimicked by the good activity of chlorophenyl ones **1e** and **2e** against most tested fungi could not be solely related to the electron withdrawing properties of Cl and NO<sub>2</sub> since analogous CF<sub>3</sub> derivatives **1h** and **2h** which carry stronger electron withdrawing CF<sub>3</sub> group (in comparison to Cl) showed low activity against all tested strains. The higher activity of **1e** and **2e** than **1h** and **2h** may be related to the higher hydrophilicity of Cl in comparison to CF<sub>3</sub> and the highest activity of nitrophenyl compounds **1g** and **2g** may be related to both hydrophilicity and electron withdrawing properties.

Next to the nitro group, significant antifungal effect was displayed by compounds substituted at the C<sup>4</sup> of the phenyl rings with heterocyclic nucleus. Of these compounds piperazinylphenyl derivative **1o** (MIC 0.07–5  $\mu\text{g/mL}$ ), ethoxycarbonyl derivative **1r** (0.3–10) and triazolyl derivative **1s** (<0.01–5) showed good to high activity against all four tested fungi; amide **1q** (<0.01–5) and phenyl derivative **1p** (0.15–10) were mostly active against *T. mentagrophytes* and *M. canis*. These findings appear to be in agreement with reports on the importance of piperazine and/or 1,2,4-triazol-3-one derivatives in azole antifungals due to their specific interaction with the heme environment of lanosterol 14 $\alpha$ -demethylase (Yoshida and Aoyama 1987) or another pharmacophoric system of fungi (Ji *et al.* 2000) as well as adjustment of the physico-chemical pro-

properties of molecule in terms of lipophilicity to avoid some toxic effects and/or to improve their pharmacokinetic properties (Sádaba *et al.* 2004). The high activity of phenyl derivatives **1c** (MIC < 0.01 µg/mL) and **2c** (0.1) against *A. niger* and their good activity against *M. canis* (0.3 and 1.5, respectively) indicate that substitution on the phenyl ring is not essential for the activity but it is beneficial.

In general, the order of the effect of substituents at C<sup>4</sup> of the phenyl ring on the antifungal activity was NO<sub>2</sub> > heterocyclic rings > Cl > H.

This class of compounds deserves further developments, since in addition of having moderate to high antibacterial effects (Khalaj *et al.* 2004) some derivatives showed high or appreciable antifungal activity.

The authors thank the *Pharmaceutical Research Centre of the Faculty of Pharmacy of Tehran Medical Sciences University* for the financial support of this investigation and Mr. Geramishoar for his excellent cooperation in antifungal screening tests.

## REFERENCES

- JANTOVÁ S., OVÁDEKOVÁ R., LETAŠIOVÁ S., ŠPIRKOVÁ K., STANKOVSKÝ Š.: Antimicrobial activity of some substituted triazoloquinazolines. *Folia Microbiol.* **50**, 90–94 (2005).
- JI H., ZHANG W., ZHOU Y., ZHANG M., ZHU J., SONG Y., LÜ J., ZHU J.: A three-dimensional model of lanosterol 14 $\alpha$ -demethylase of *Candida albicans* and its interaction with azole antifungals. *J.Med.Chem.* **43**, 2493–2505 (2000).
- KHALAJ A., ADIBPOUR N., SHAHVERDI A.R., DANESH TALAB M.: Synthesis and antibacterial activity of 2-(4-substituted phenyl)-3(2H)-isothiazolones. *Eur.J.Med.Chem.* **43**, 699–705 (2004).
- LEWIS S.N., MILLER G.A., LAW A.B.: Pesticidal substituted 3-isothiazolinones. *French Pat.* 1 555 416 (1969); *Chem.Abstr.* **72**, 111 459n (1970).
- LEWIS S.N., MILLER G.A.: Biocidal acyl derivatives of oxo- and oxyisothiazoles. *US Pat.* 3 544 580 (1970); *Chem.Abstr.* **76**, 34 242q (1972).
- LEWIS S.N., MILLER G.A.: 3-Hydroxyisothiazole and 4-isothiazolinone-3-on derivatives. *US Pat.* 3 706 757 (1972); *Chem.Abstr.* **78**, 84 399hq (1973).
- LEWIS S.N., MILLER G.A., LAW A.B.: 3-Isouthiazolones. *US Pat.* 3 761 488 (1973).
- LEWIS S.N., MILLER G.A.: Derivatives of 3-isouthiazolones with active halogen compounds. *US Pat.* 3 835 150 (1974); *Chem.Abstr.* **81**, 152 212f (1974).
- LEWIS S.N., MILLER G.A., LAW A.B.: 3-Isouthiazolones as biocides. *US Pat.* 4 105 431 (1978); *Chem.Abstr.* **90**, 54 934j (1979).
- LEWIS S.N., MILLER G.A., LAW A.B.: Cosmetic formulation comprising 3-isouthiazolones. *US Pat.* 4 265 899 (1981).
- MESSICK C.R., PENDLAND S.L., MOSHIRFAR M., FISCELLA R.G., LOSENDAHL K.J., SCHRIEVER C.A., SCHRECKENBERGER P.C.: *In-vitro* activity of polyhexamethylene biguanide (PHMB) against fungal isolates associated with infective keratitis. *J.Antimicrob. Chemother.* **44**, 297 (1999).
- SÁDABA B., GARCÍA-QUETGLAS E., AZANZA J.R.: Relación entre e estructura y función en los azoles. *Rev.Esp.Quimioterap.* **17**, 71–78 (2004).
- YOSHIDA Y., AOYAMA Y.: Interaction of azole antifungal agents with cytochrome P-45014DM purified from *Saccharomyces cerevisiae* microsomes. *Biochem.Pharmacol.* **36**, 229–235 (1987).