Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1893–1895

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

3-Phenyl-5-methyl-2*H*,5*H*-furan-2-ones: Tuning Antifungal Activity by Varying Substituents on the Phenyl Ring

Milan Pour,[a,*] Marcel Špulák,[a] Vojtěch Balšánek,[a] Jiří Kuneš,[a] Vladimír Buchta[b] and Karel Waisser[a]

[a]*Laboratory of Structure and Interactions of Biologically Active Molecules, Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, CZ-500 05 Hradec Králové, Czech Republic*
[b]*Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Heyrovského 1203, CZ-500 05 Hradec Králové, Czech Republic*

Received 16 May 2000; revised 26 June 2000; accepted 27 June 2000

Abstract—A series of racemic 3-phenyl-5-methyl-2*H*,5*H*-furan-2-ones related to a natural product, (−)incrustoporine, was synthesized, and their antifungal activity evaluated. The key structural feature, furanone ring, was closed via H_2SO_4-mediated cyclization of 2-phenylpent-4-enoic acids. The compounds displayed antifungal activity, especially against filamentous fungi. Expressed as the minimum inhibition concentration (MIC) in μmol/L, the activity of the most promising derivative against *Absidia corymbifera* matched that of ketoconazole (31.25 μmol/L). In terms of μg/mL, the substance was more active (7.6 μg/mL) than this standard antifungal drug (16.6 μg/mL).

As there are just a few structurally different groups of antifungal agents, which is especially true about the systemic ones,[1] identification of new lead structures and further development of novel antifungal drugs is an important goal of current pharmaceutical research. In particular, it appears highly desirable to continue the process of drug discovery for the treatment of serious mycoses caused by opportunistic fungal pathogens, such as the members of *Mucorales*, *Aspergillus*, *Fusarium*, *Trichosporon* and non-*albicans Candida* species, which are characterized with decreased susceptibility to current antifungal drugs.[2]

In 1995, Zapf et al. reported[3] the isolation of an interesting fungal metabolite from the family of butenolides, (−)incrustoporine **6**. The natural product was found to possess activity against a wide array of phytopathogenic fungi as well as some cytotoxic activity.[3] Following the isolation, two total syntheses were published, by Yajima and Mori[4] in 1996, and later by Rossi[5] in 1999. The structure of the compound indicates that it is a relatively lipophilic, small, rigid molecule of low structural complexity, which renders it a possible target for further development as a potential drug. As part of a medicinal chemistry program focused on the search for new antimycotic agents based on optimizing natural product lead structures, we were interested in preparing simple analogues of (−)incrustoporine. We speculated that, similar to griseofulvin,[6] compounds related to (−)incrustoporine could also display activity against pathogenic fungi targeting humans as their hosts. In this letter, we wish to disclose the design, preparation and biological evaluation of a series of 3-phenyl-5-methyl-2*H*,5*H*-furan-2-ones, the goal of which was a preliminary assessment of the suitability of this class of compounds for further development as potential antimycotics.

At the initial stage of our research, we aimed to prepare compounds with various substituents with different electronic influence on the phenyl ring, and a simple alkyl moiety at C(5) of the furanone ring. In the natural product lead, the ethyl group at C(5) is attached to a centre of chirality. To introduce this stereochemical element, Yajima and Mori[4] used a rather costly 1,(2*S*)-epoxybutane in a moderate-yielding epoxide ring opening step (53%) of their sequence. As the possibility of biological evaluation against human pathogenic fungi providing negative results (and thus adding little value to the synthetic endeavours) could not be ruled out, we designed a series of simple 5-methyl derivatives **5**. We envisaged an easy, high-yielding route to these compounds from their saturated precursors **4**, which in turn could be easily derived from the cyclization of 2-phenylpent-4-enoic acids **3** with different substituents attached to the phenyl ring (Fig. 1).

*Corresponding author. Fax: +42-49-5210002; e-mail: pour@faf.cuni.cz

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00376-0

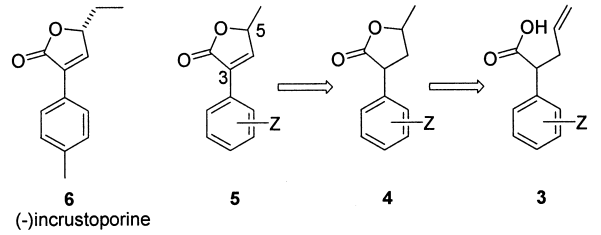
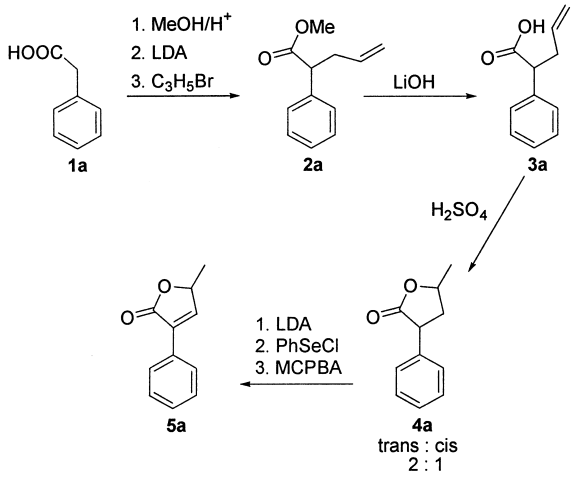


Figure 1.

The preparation of the unsubstituted 3-phenyl-5-methyl-2*H*,5*H*-furan-2-one **5a** is outlined as a representative example in Scheme 1. Thus, phenylacetic acid was first converted into the corresponding methyl ester. Following the deprotonation with LDA, the ester enolate was quenched with allyl bromide to afford the methyl ester of 2-phenylpent-4-enoic acid. The carboxylic group was then liberated by hydrolysis, and subjected to a proton-mediated cyclization onto the terminal double bond to afford both diastereomers of 3-phenyl-5-methyltetrahydrofuran-2-one. Following some experimentation, we found that the reaction proceeded best in concentrated sulphuric acid at 0 °C, giving almost quantitative yield of a mixture of *trans* and *cis* isomers in the ratio of 2:1, as determined by NMR. Finally, the double bond was introduced by enolization of the mixture of saturated lactones followed by treatment with phenylselenenyl chloride, oxidation and spontaneous selenoxide elimination to yield the target compound. Throughout the sequence, yields in all steps did not fall below 80% for each target molecule.

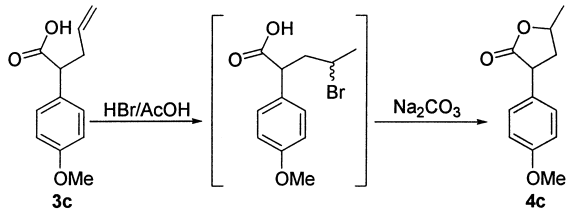


Scheme 1.

A minor modification had to be used in the preparation of the *p*-methoxy derivative **5c**, as its saturated precursor **4c** could not be prepared by the sulphuric acid induced cyclization of 2-(*p*-methoxyphenyl)pent-4-enoic acid **3c** due to the competing sulfonation of the phenyl ring.

Acid **3c** was therefore subjected to reaction with hydrogen bromide, and the intermediate bromide was immediately cyclized with sodium carbonate in methanol (Scheme 2).

All unsaturated lactones **5a–j**[7–9] prepared via the above procedures are summarized in Table 1.



Scheme 2.

The target compounds and their saturated precursors were evaluated for their in vitro antifungal activity against a set of eight clinical isolates of human pathogenic fungi (*Candida albicans* ATCC 44859, *Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Trichosporon beigelii* 1188, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445) using the microdilution format of the NCCLS M27-A guidelines.[10] In order to have a standard for comparison, we also synthesized natural incrustoporine in the racemic form by the route of Yajima and Mori,[4] and ketoconazole was used as a standard antifungal drug. The saturated lactones **4a–j** displayed no antifungal activity at all (>1000 μmol/L), but the target compounds **5a–j** and the racemic natural product **6** were found to be antifungally active in general. While the activity of racemic incrustoporine itself was moderate to low, and the compound was only marginally more efficient than its 5-methyl counterpart **5b**, the halogenated analogues displayed appreciable fungistatic effect, in particular against filamentous fungi (Table 1).

The activity of the most potent 3-(3,4-dichlorophenyl)-5-methyl-2*H*,5*H*-furan-2-one **5e** against the strains of *Aspergillus fumigatus* (AF) and *Absidia corymbifera* (AC) read after 24 h is higher than that of ketoconazole. After 48 h, MIC of **5e** against AF increases as compared to ketoconazole, but the compound remains as efficient as this antifungal drug against AC. Expressed in μg/mL, even after the period of 48 h, furanone **5e** is more active against AC (7.6 μg/mL) than ketoconazole (16.6 μg/mL). The activity of the substances against yeast strains is not so significant, and generally increases with time.

In conclusion, the results show that the structure of (−)incrustoporine represented by the unsaturated five-membered lactone ring may serve as a potential lead to the preparation of analogues possessing antifungal activity against human pathogens. Antifungal activity appears to be linked to the presence of the double bond conjugated with the carbonyl group of the lactone function, and can be significantly increased by substituting the phenyl ring with halogens. Encouraged by these results, we are continuing to explore this class of antifungal compounds and our progress will be reported in due course.

Table 1. In vitro antifungal activities of compounds **5a–j**, (±)**6** and ketoconazole (**k**)

Strain	Time (h)	Compound No. — MIC (μmol/L)[a]											
		5a H	**5b** *p*-Me	**5c** *p*-MeO	**5d** *p*-Cl	**5e** *m*,*p*-Cl	**5f** *p*-F	**5g** *m*-F	**5h** *p*-Br	**5i** *m*-Br	**5j** *p*-NO_2	**6**	**k**
TM[b]	72	500	250	125	31.25	31.25	250	125	31.25	62.5	62.5	125	0.98
	120	1000	500	250	62.5	62.5	250	125	62.5	125	125	250	1.95
CA	24	1000	1000	1000	31.25	31.25	62.5	62.5	7.81	31.25	250	250	0.12
	48	>1000	>1000	1000	125	500	250	250	62.5	125	500	500	0.12
CT	24	>1000	>1000	1000	250	>500	250	500	250	250	500	500	15.63
	48	>1000	>1000	>1000	500	>500	500	1000	500	500	500	>500	15.63
CK	24	>1000	>1000	>1000	250	>500	500	500	250	250	500	500	3.91
	48	>1000	>1000	>1000	500	>500	500	1000	250	500	500	>500	3.91
CG	24	>1000	>1000	>1000	500	>500	500	>1000	500	250	500	500	0.24
	48	>1000	>1000	>1000	1000	>500	500	>1000	1000	500	1000	>500	0.49
TB	24	>1000	>1000	1000	125	31.25	125	250	125	62.5	500	250	0.12
	48	>1000	>1000	>1000	250	>500	250	500	250	250	1000	500	0.12
AF	24	1000	1000	1000	62.5	7.81	125	250	62.5	62.5	250	250	15.63
	48	>1000	>1000	1000	125	62.5	125	500	250	250	1000	500	15.63
AC	24	1000	1000	1000	125	7.81	62.5	500	125	62.5	250	250	31.25
	48	>1000	>1000	>1000	250	31.25	62.5	500	125	125	250	250	31.25

[a]Minimum inhibitory concentration.
[b]TM = *Trichophyton mentagrophytes*; CA = *Candida albicans*; CT = *Candida tropicalis*; CK = *Candida krusei*; CG = *Candida glabrata*; TB = *Trichosporon beigelii*; AF = *Aspergillus fumigatus*; AC = *Absidia corymbifera*.

Acknowledgements

This work was financially supported by Charles University (project No. 171/1997/B) and by the Ministry of Education of the Czech Republic (project No. VS 97124 and LB 98233). The following undergraduate students participated in this project: Petr Koudelka, Veronika Myšáková, Radan Schiller, Hana Šebestiánová and Filip Št'astný. The mass spectra were recorded by Dr. V. Voříšek, Department of Clinical Biochemistry, Charles University Medical Faculty Hospital, Hradec Králové, Czech Republic.

References and Notes

1. Kaufman, C. A.; Carver, P. L. *Drugs* **1997**, *53*, 539.
2. Graybill, J. R. *Clin. Infect. Dis.* **1996**, *22* (Suppl. 2), S166.
3. Zapf, S.; Anke, T.; Sterner, O. *Acta Chem. Scand.* **1995**, *49*, 233.
4. Yajima A., Mori K. *Liebigs Ann. Chem.* **1996**, 1091.
5. Rossi, R.; Bellina, F.; Biagetti, M.; Mannina, L. *Tetrahedron: Asymmetry* **1999**, *10*, 1163.
6. De Carli, L.; Larizza, L. *Mutation Research* **1988**, *195*, 91.
7. Compound **5a** was described by DeShong and co-workers: DeShong, P.; Sidler, D. R.; Rybczynski, P. J.; Slough, G. A.; Rheingold, A. L. *J. Am. Chem. Soc.* **1988**, 110, 2575.
8. Compounds **5b**, **5c**, **5d** and **5j** were reported to have been obtained by the decomposition of the corresponding manganacycles: DeShong, P.; Sidler, D. R.; Rybczynski, P. J.; Ogilvie, A. A. *J. Org. Chem.* **1989**, 54, 5432. However, neither the yields, nor the spectral data and physical constants are presented in this paper.
9. All compounds were fully characterized by spectral methods, and their purity checked by elemental analysis. Representative data of the most potent derivative: compound **5e**: mp 108–111 °C; IR ($CHCl_3$) ν_{max} 1323, 1472, 1758, 2988, 3026 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.99 (1H, d, J = 1.9 Hz, Ar), 7.73 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.9 Hz, Ar), 7.60 (1H, d, J = 1.9 Hz, H4), 7.48 (1H, d, J = 8.4 Hz, Ar), 5.17 (1H, qd, J_1 = 6.9 Hz, J_2 = 1.9 Hz, H5), 1.53 (3H, d, J = 6.9 Hz, $-CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.71, 150.15, 133.18, 132.65, 130.39, 129.17, 128.95, 128.61, 126.05, 76.87, 18.98; LRMS: 242 ($M^{\bullet+}-H$, 100), 225 (4), 214 (3), 199 (33), 171 (94), 150 (7), 136 (31), 126 (7), 115 (9), 99 (13), 86 (6), 74 (13), 63 (9), 50 (14). Anal. calcd for $C_{11}H_8Cl_2O_2$: C, 54.35; H, 3.32. Found: C, 54.56; H, 3.25.
10. National Committee For Clinical Laboratory Standards. (1997). Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A. NCCLS 1997, Villanova, PA.