Bioorganic & Medicinal Chemistry Letters 20 (2010) 7358–7360

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# 3,5-Disubstituted pyranone analogues of highly antifungally active furanones: Conversion of biological effect from antifungal to cytostatic

Radan Schiller [a], Lucie Tichotová [a], Jan Pavlík [a], Vladimír Buchta [b], Bohuslav Melichar [c], Ivan Votruba [d], Jiří Kuneš [a], Marcel Špulák [a], Milan Pour [a,*]

[a] Centre For New Antivirals and Antineoplastics, Department of Inorganic and Organic Chemistry, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, CZ-500 03 Hradec Králové, Czech Republic
[b] Department of Clinical Microbiology, University Hospital and Charles University, Faculty of Medicine, Sokolská 581, CZ-500 03 Hradec Králové, Czech Republic
[c] Institute of Experimental Neurosurgery, University Hospital and Charles University, Sokolská 581, CZ-500 03 Hradec Králové, Czech Republic
[d] Institute of Organic Chemistry and Biochemistry, Flemingovo n. 2, CZ-166 10 Prague 6, Czech Republic

ARTICLE INFO

*Article history:*
Received 24 August 2010
Revised 12 October 2010
Accepted 13 October 2010
Available online 21 October 2010

*Keywords:*
Pyranone
Synthesis
Screening
Antifungal
Cytostatic

ABSTRACT

A series of 3-aryl-5-acyloxymethyl-5,6-dihydro-2*H*-pyran-2-ones, related to highly antifungally active butenolides, was synthesized via cyclization of substituted δ-hydroxy acids as the key step, and evaluated for their in vitro antifungal activity and cytostatic activity. While the extension of the furanone ring to pyranone led to a complete loss of the antifungal effect, some of the compounds displayed promising effect against several cell lines, including the resistant colorectal carcinoma cells.



Several years ago, we disclosed antifungal analogues of natural 3,5-disubstituted butenolides,[1–4] the structure–activity relationships of which clearly indicated that the necessary pharmacophoric features include: (1) double bond in conjugation with the lactone moiety and (2) halogenated phenyl ring at C(3). The in vitro antifungal effect of the most successful series, that is, 3-(halogenated phenyl)-5-acyloxymethyl-2,5-dihydrofuran-2-ones (**1**, Fig. 1), was comparable to that of amphotericin B, especially against the dangerous filamentous human pathogen *Aspergillus fumigatus*. A flow cytometric study[5] revealed that the compounds of this series damage the fungal cell membrane of *Candida albicans*. On the molecular level, the SARs of the furanones[1,3,6] together with zero activity of related 3,6-disubstituted pyranones[7] have clearly indicated that a non-specific Michael addition of a nucleophile to the β-carbon of the butenolide ring is an unlikely mechanism of action. Very recently, we disclosed the finding[6] that the nature of antifungal action of compounds **1** lies in the ability of some of the derivatives (Z = 4-Br, 3,4-diCl) to produce the corresponding, highly antifungally active 5-methylene furanones **2** upon dissolution in DMSO under antifungal assay conditions (Fig. 1).

Further development of the furanone pharmacophore led to the ring expansion into pyranone, with the acyloxymethyl moiety being located at C6 (**3**). Surprisingly, the resultant pentenolides showed total lack of both antifungal activity and cytostatic effect.[7] Nonetheless, another possible location of the acyloxymethyl group would logically be C5, leading onwards to 3,5-disubstituted-5,6-dihydro-2*H*-pyran-2-ones (**4**). In this communication, we report the synthesis and biological evaluation of these compounds, which

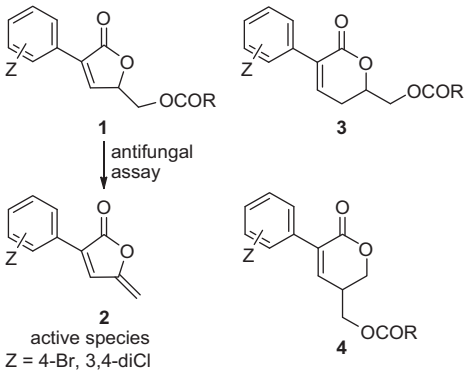



**Figure 1.** Design of the title pyranones **4**.

* Corresponding author. Tel.: +420 49 5067277; fax: +420 49 5067166.
*E-mail address:* milan.pour@faf.cuni.cz (M. Pour).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.10.052

may serve as novel natural product-derived leads in anticancer drug development.

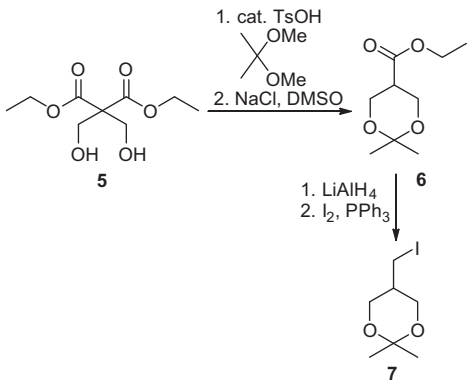
While a number of inventive approaches towards 6-substituted pentenolides have appeared in the literature over the past 10 years,[8] 3,5-disubstituted compounds received practically no attention. Thus, because of the simplicity of the methodology as well as commercial availability of substituted phenylacetic acids, we employed a proven cyclization strategy which had been successfully applied to the synthesis of 3,5-disubstituted-2,5-dihydrofuran-2-ones,[1–3] and recently in the construction of the 3,6-disubstituted pentenolide ring.[7] Hence, given the structure of the title compounds **4**, the sequence is based on α-alkylation of protected phenylacetic acids with a suitably substituted alkylating agent followed by cyclization and introduction of the double bond into the ring.

As regards the alkylating agent, 5-iodomethyl-2,2-dimethyl-1,3-dioxan (**7**), obtainable from the commercially available diethyl-bis(hydroxymethyl)malonate **5** (Scheme 1) over four steps[9] was a convenient choice.

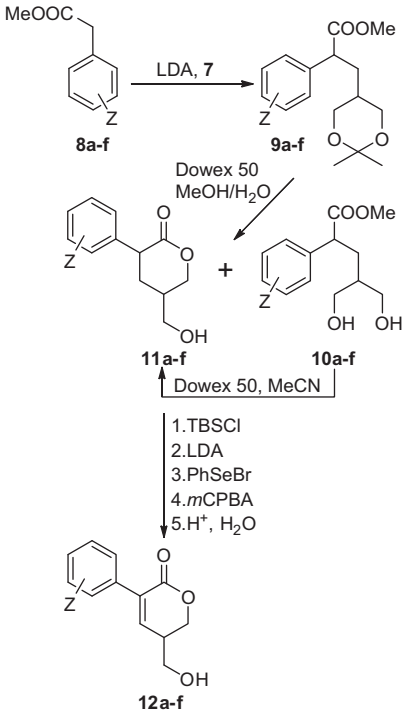
The preparation of the key intermediates, 3-aryl-5-hydroxymethyl-5,6-dihydro-2*H*-pyran-2-ones (**12**) is outlined in Scheme 2. Thus, methyl esters of substituted phenylacetic acids (**8a**–**f**) were deprotonated with LDA, and the enolates quenched with iodide **7** to afford the methyl esters of 2-aryl-3-(2,2-dimethyl-1,3-dioxan-5-yl)propanoic acids (**9a**–**f**). Even though a spontaneous cyclization to six-membered lactones following the deprotection of esters **9a**–**f** with Dowex 50 in MeOH/$H_2O$ could be expected, we obtained a mixture of the desired products together with acyclic material in approximately 1:1 ratio. Fortunately, the cyclization could be driven to completion[7] by replacing the solvent with MeCN. In order to introduce the double bond, the hydroxy group was protected as a TBS ether, the product enolized with LDA and the enolate quenched with PhSeBr. The intermediate 3-phenylselanyl derivatives were, due to their limited stability, immediately oxidized to afford the corresponding selenoxides, which underwent a spontaneous *syn*-elimination. Subsequent acidic removal of the silyl group then furnished the target pyranone alcohols **12a**–**f**.

Finally, alcohols **12a**–**f** were treated with selected acyl chlorides under mild conditions[2] in the presence of pyridine (Scheme 3).

All target esters **4ax**–**fz** were evaluated for their in vitro antifungal activity against a set of human pathogenic fungi including the representatives of both yeast (*C. albicans* ATCC 44859, *C. albicans* ATCC 90028, *Candida krusei* ATCC 6258, *C. krusei* E28, *Candida parapsilosis* ATCC 22019, *Candida glabrata* 20/I, *Candida tropicalis* 156, *Candida lusitaniae* 2446/I, and *Trichosporon beigelii* 1188) and filamentous strains (*A. fumigatus* 231, *Absidia corymbifera* 272, *Microsporum gypseum*, and *Trichophyton mentagrophytes* 445) using the microdilution format of the NCCLS M27-A guidelines.[10] In general, the antifungal effect of pyranones **4** was low to negligible

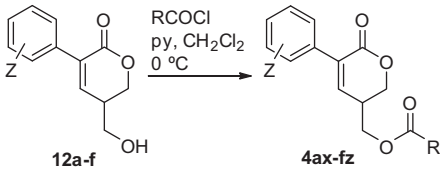



**Scheme 1.** Synthesis of alkylating agent **7**.




**a** Z = H; **b** Z = 4-Br; **c** Z = 3,4-$Cl_2$;
**d** Z = 3-Cl; **e** Z = 4-Cl; **f** Z = 4-OMe

**Scheme 2.** Synthesis of pyranone alcohols **12**.




**a** Z = H; **b** Z = 4-Br; **c** Z = 3,4-$Cl_2$;
**d** Z = 3-Cl; **e** Z = 4-Cl; **f** Z = 4-OMe
**x:** R = $CH_3$; **y**: R = $(CH_3)_3C$;
**z**: R = phenyl

**Scheme 3.** Preparation of pyranone esters **4**.

(MIC values $\geqslant$62.5 μmol $L^{-1}$), and there appeared to be no significant differences in the activity of the phenyl derivatives (**4ax**–**4az**), *p*-methoxyphenyl derivatives (**4fx**–**4fz**), and the halophenyl compounds (**4bx**–**4bz**, **4cx**–**4cz**, **4dx**–**4dz**, and **4ex**–**4ez**). Importantly, unlike the analogous furanone esters[6] (**1**), pyranone esters **4** showed no sign of an easy elimination to furnish the corresponding 5-methylene derivatives analogous to **2** (see Fig. 1). For example, the solution of pentenolide **4ax** has been stable in DMSO-$d_6$ solution for an indefinitely long time, as evidenced by NMR. Also, in order to prepare the corresponding 5-methylene compound from derivative **12a**, alcohol **12a** had to be tosylated and the tosylate treated with DBU.

However, initial screening for cytostatic activity[11,12] on mouse lymphocytic leukemia L1210 cells (ATCC CCL 219), CCRF-CEM T lymphoblastoid cells (human acute lymphoblastic leukemia, ATCC CCL 119), human promyelocytic leukemia HL-60 cells (ATCC CCL 240), and human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2) revealed potentially interesting activity of compounds **4cx**, **4cz**, and **4ez** at the concentration of 10 μmol $L^{-1}$. While all other derivatives displayed marginal or no inhibition of cell growth at this concentration, these compounds substituted with chlorine on the phenyl

**Table 1**
Growth inhibitory activity ($IC_{50}$, μmol $L^{-1}$) of compounds **12c**, **4cx**, **4cz**, and **4ez**

| Cell line | Compound | | | |
|---|---|---|---|---|
| | **12c** | **4cx** | **4cz** | **4ez** |
| L1210 | NA[a] | 7.3 ± 0.42 | 5.9 ± 0.35 | NA |
| HL-60 | NA | 7.3 ± 0.44 | 6.1 ± 0.36 | NA |
| HeLa S3 | NA | 6.8 ± 0.41 | 6.7 ± 0.40 | NA |
| CCRF-CEM | NA | 3.6 ± 0.26 | 4.5 ± 0.27 | 7.1 ± 0.50 |

[a] Not active at relevant concentrations.

**Table 2**
Comparison of growth inhibitory activity ($IC_{50}$, μmol $L^{-1}$) of compounds **12c**, **4cx**, and **4ez** and two drug standards (**OxPt**—oxaliplatin, **Irt**—irinotecan)

| Cell line | Compound | | | | |
|---|---|---|---|---|---|
| | **12c** | **4cx** | **4ez** | **OxPt** | **Irt** |
| HT-29 | NA | 2.45 ± 0.15 | 2.93 ± 0.18 | NA | 4.73 ± 0.28 |

ring and easy-to-hydrolyze acyl group showed a promising inhibitory effect. The determination of their $IC_{50}$ values (Table 1) revealed that they were in the range of 3–7 μmol $L^{-1}$.

Selected compounds (**12c**, **4cx**, and **4ez**) were subsequently subjected to screening against colorectal carcinoma cell line HT-29 (ATCC HTB 38), resistant to most cytostatic agents. Among the three drugs currently used in therapy of colorectal carcinoma (5-fluorouracil, irinotecan, and oxaliplatin), HT-29 cells are moderately sensitive only to irinotecan. Thus, the low $IC_{50}$ values observed in HT-29 cells (Table 2) are of particular interest. It is also worthy to note that the 4-chlorophenyl derivative **4ez** showed interesting activity only against the CCRF-CEM and HT-29 cells, which might be a sign of possible selectivity.

Because all pyranones **4** can act as Michael acceptors, derivative **4ez** was subjected to a reaction with PrSH in THF–$H_2O$ and the presence of $K_2CO_3$. While no conjugate addition was observed at 0 °C, less than 5% of the addition product was detected in the $^1H$ NMR spectrum of the crude reaction mixture after 5 h at rt.[13] Apparently, the 3-arylated pyranones do not easily undergo conjugate addition of a thiolate.

In summary, we have prepared a series of 3-aryl-5-acyloxymethyl-5,6-dihydro-2*H*-pyran-2-ones,[14] derived from the structure of highly antifungally active furanones. Similar to the recently described[7] 6-acyloxymethyl analogues, this change led to a complete loss of antifungal activity, which is thus specifically linked to furanone (butenolide) ring. Most noteworthy, some of the 3,5-disubstituted pentenolides showed promising cytostatic activity. While it could be argued that a non-specific conjugate addition might play a role in the cytostatic effect, it should be noted that the compounds do not easily undergo the addition of a nucleophile as strong as a thiolate. Especially remarkable was the activity against the resistant colorectal carcinoma cells, which renders the substances possible leads for further investigation and development as potential anticancer agents. Despite recent progress of combination chemotherapy and introduction of biological agents, such as bevacizumab and cetuximab, more active agents are urgently needed for the therapy of colorectal carcinoma.

## Acknowledgments

This work was supported by Charles University (project No. SVV/2010/261/001) and the Ministry of Education, Youth and Sports of the Czech Republic (projects Nos. 1M0508 and MSM0021620822).

## References and notes

1. Pour, M.; Špulák, M.; Balšánek, V.; Kuneš, J.; Buchta, V.; Waisser, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1893.
2. Pour, M.; Špulák, M.; Buchta, V.; Kubanová, P.; Opršalová, M.; Wsól, V.; Fáková, H.; Koudelka, P.; Pourová, H.; Schiller, R. *J. Med. Chem.* **2001**, *44*, 2701.
3. Pour, M.; Špulák, M.; Balšánek, V.; Kuneš, J.; Kubanová, P.; Buchta, V. *Bioorg. Med. Chem.* **2003**, *11*, 2843.
4. Buchta, V.; Pour, M.; Kubanová, P.; Silva, L.; Votruba, I.; Opršálová, M.; Schiller, R.; Fáková, H.; Špulák, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 873.
5. Vale-Silva, L. A.; Buchta, V.; Vokurková, D.; Pour, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2492.
6. Šenel, P.; Tichotová, L.; Votruba, I.; Buchta, V.; Špulák, M.; Kuneš, J.; Nobilis, M.; Krenk, O.; Pour, M. *Bioorg. Med. Chem.* **2010**, *18*, 1988.
7. Šnajdr, I.; Pavlík, J.; Schiller, R.; Kuneš, J.; Pour, M. *Collect. Czech. Chem. Commun.* **2007**, *72*, 1472.
8. For an up-to-date review on novel strategies, see: Boucard, V.; Broustal, G.; Campagne, J. M. *Eur. J. Org. Chem.* **2007**, 225.
9. Iwata, C.; Fujita, M.; Moritani, Y.; Sugiyama, K.; Hattori, K.; Imanishi, T. *Tetrahedron Lett.* **1987**, *28*, 3131.
10. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved Standard, NCCLS document M27-A, 771 E. Lancaster Avenue, Villanova, PA, 19085, 1997.
11. Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817.
12. Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
13. The rest was unchanged starting material.
14. *Analytical data for 5-acetyloxymethyl-3-(3,4-dichlorophenyl)-5,6-dihydro-2H-pyran-2-one* (**4cx**): white crystals, mp 75–78 °C; $^1H$ NMR: (300 MHz, $CDCl_3$) $\delta$ 7.58 (1H, d, $J$ = 2.2 Hz, ArH2), 7.45 (1H, d, $J$ = 8.5 Hz, ArH5), 7.33 (1H, dd, $J_1$ = 2.2 Hz, $J_2$ = 8.5 Hz, ArH6), 6.91 (1H, d, $J$ = 4.4 Hz, H4), 4.58–4.51 (1H, m, H6), 4.45–4.37 (1H, m, H6), 4.28 (1H, dd, $J_1$ = 5.5 Hz, $J_2$ = 11.3 Hz, $CH_2O$), 4.19 (1H, dd, $J_1$ = 7.4 Hz, $J_2$ = 11.3 Hz, $CH_2O$), 3.10–2.98 (1H, m, H5), 2.10 (3H, s, $CH_3COO$); $^{13}C$ NMR: (75 MHz, $CDCl_3$) $\delta$ 170.6, 162.5, 141.8, 134.7, 133.0, 132.5, 132.3, 130.6, 130.2, 127.7, 67.9, 62.2, 34.6, 20.7; IR: ($CDCl_3$) $\nu_{max}$ 2956 (w), 2899 (w), 1731 (s), 1471 (m), 1366 (m) $cm^{-1}$; LRMS (ESI): $m/z$ (relative intensity) 315 $[M+H]^+$ (2), 281 (8), 265 (7), 247 (5), 235 (49), 221 (12), 207 (8), 191 (4), 173 (100), 158 (15), 147 (87), 134 (20), 121 (25), 105 (21), 91 (30), 73 (75), 59 (20); C,H,N: calcd for $C_{14}H_{12}Cl_2O_4$: C, 53.36; H, 3.84; found: C, 53.35; H, 4.12.