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Original article

Synthesis and evaluation of cytotoxic activity of arylfurans

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Abstract

A series of 2-aryl and 2,5-diarylfurans were synthesized and evaluated for their in vitro cytotoxicity against human cancer cells lines and on the proliferation of human peripheral blood mononuclear cells (PBMC) stimulated with phytohemaglutinin (PHA). Three compounds were found to present significant activity against the cancer cell lines without affecting the lymphocyte proliferation in PBMCs, indicating low toxicity to normal cells.

Keywords: Arylfurans; Cytotoxic activity; Cancer cells; PBMC

1. Introduction

Tetrahydrofuran lignans and their analogues (Fig. 1) display a large range of biological effects including neuroprotection [1], inhibition of nitric oxide synthesis [2], antiplasmodial [3], antifungal [4], and trypanocidal [5] activities, platelet-activating factor (PFA) antagonism [6,7].

Recently, 2,5-diaryl-3,4-dimethyltetrahydrofuran lignans **1**–**3** (Fig. 2) were tested on lymphocyte proliferation, growth of tumor cell, and complement activation, showing moderate activities in the first ($IC_{50} \geq 84$ μM) and the second models ($GI_{50} \geq 33$ μM) [8].

Encouraged by these results and aiming at more active compounds, we decided to evaluate the cytotoxic activity of 2,5-diarylfurans containing different number of methoxy groups attached to the aryl ring (compounds **4**–**7**), designed as molecular simplifications of the tetrahydrofuran lignans. During this exercise, mono- and diarylfurans bearing others substituents on the phenyl ring were prepared (compounds **8**–**16**), in order to study the influence of different substituents on the activity (Fig. 3).

2. Chemistry

The arylfurans **4**–**16** were synthesized using methods previously reported by us [9] and outlined in Fig. 4.

A simple, rapid and mild method for the direct arylation of furans consists in the well-known Meerwein reaction (copper

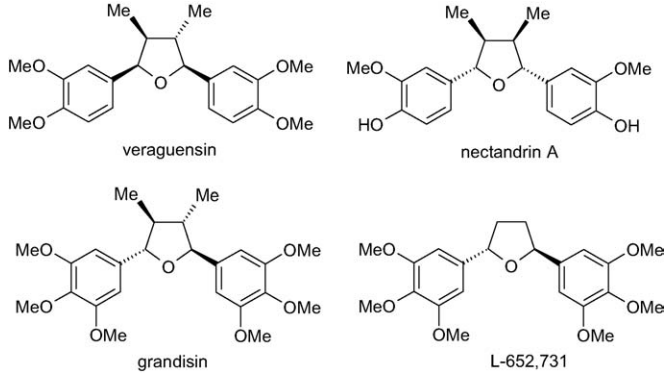

Fig. 1. Chemical structures of tetrahydrofuran lignans and the corresponding *trans* desmethyl analogue L-652,731.

Abbreviations: PBMC, peripheral blood mononuclear cells; PHA, phytohemaglutinin.

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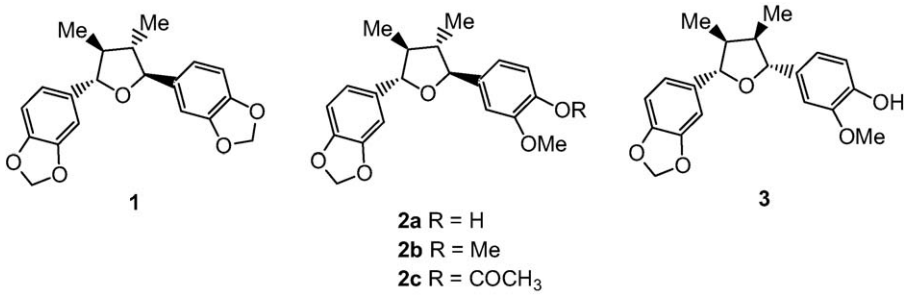

Fig. 2. Chemical structures of tetrahydrofuran lignans tested on lymphocyte proliferation, growth of tumor cell, and complement activation.

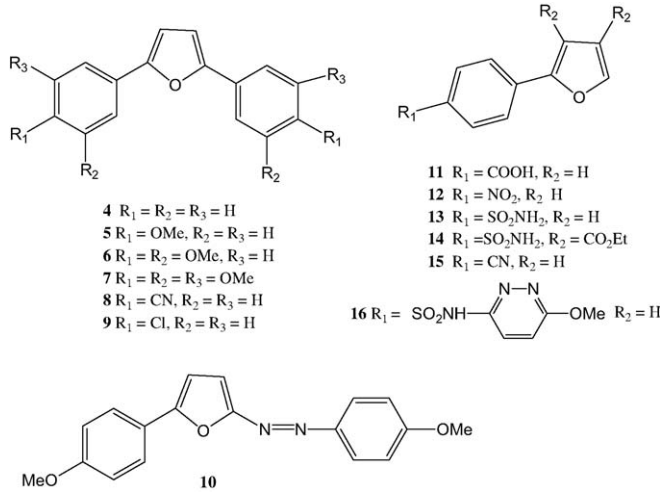

Fig. 3. Chemical structures of compounds prepared in this work.

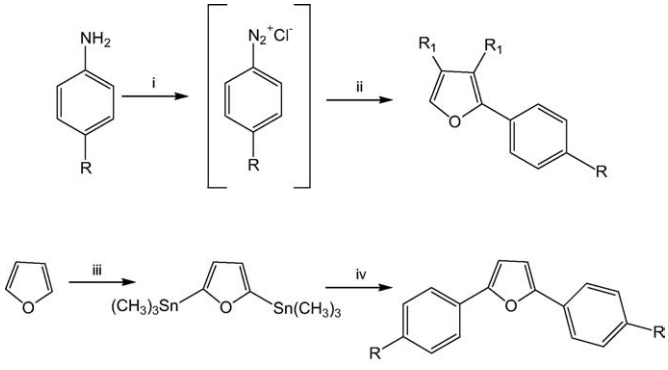

Fig. 4. *Reagents and conditions*: i) HCl, $NaNO_2$, H_2O, then ii) acetone, $CuCl_2{\cdot}2H_2O$, furan, r.t., 12–48%; iii) *n*-BuLi, TMDA, $(CH_3)_3SnCl$, hexane, r.t., 42%; iv) $Pd(PPh_3)_4$, arylhalides, dioxane, 85 °C, 22–40%.

(II) salts catalyzed arylation of olefin compounds) [10]. The availability and low cost of aromatic amines and furan frequently offset the low yields obtained using this methodology. However, such reaction was applicable neither to the preparation of the diarylfurans nor to monoarylfurans having electron donor substituents attached to the phenyl ring. For example, the reaction of furan with *p*-methoxyaniline furnished only tarry material from which the unexpected azofuran **10** was isolated in low yield. As 2,5-diphenylfurans have already been prepared in two steps *via* Stille coupling reaction [11], we decided to use this methodology to prepare the substituted diarylfurans.

3. Pharmacology

Compounds **4**–**16** were assayed in vitro at 20 μg/ml (50–120 μmol/l) against the human cancer cells lines MCF-7 (breast), TK-10 (renal) and UACC-62 (melanoma) and on normal human peripheral blood mononuclear cells (PBMC) stimulated with phytohemaglutinin (PHA), using etoposide and dexamethasone as standard control drugs, respectively.

The results are expressed as percentage of inhibition of the growth of the three tumor cell lines after treatment with each compound, using the sulforhodamine B (SRB) protein assay to estimate cell growth [12].

The in vitro PBMC assay was performed as previously described in [13] and proliferation evaluated by MTT method [14]. This assay provides clues for possible toxic effects on normal human cells under rapid proliferation [15].

The concentrations that cause 50% inhibition of the tumor cell growth (GI_{50}) and 50% inhibition of PBMC proliferation (IC_{50}) were evaluated for the more potent compounds.

4. Results and discussion

Comparative cytotoxicity of arylfurans against the human cancer cells lines and human PBMC was investigated and the results are summarized in Table 1. The ability to inhibit the growth of cancer cells without affecting lymphocyte proliferation in PBMC is a good indication of selectivity of the compound, a desirable property for a drug candidate in cancer chemotherapy.

The compounds **6**, **10**, and **12**, were found to be toxic at 20 micrograms/mL against one or more of the cancer cells lines used, with the mean growth inhibition above 80%. The other compounds were considered inactive at this dose. It is interesting to note that while the dimethoxy derivative **6** was active against all three lines, the mono methoxy compound **5** (4-OMe) and trimethoxy **7** (3,4,5-OMe) were practically inactive. The concentrations that cause 50% inhibition of the tumor cell growth (GI_{50}) and 50% inhibition of PBMC proliferation (IC_{50}) were evaluated for the most active compounds (**6**, **10** and **12**) and are summarized in Table 2. The results revealed the interesting selectivity presented for compounds **10** and **12**, which were much more toxic to the breast cancer MCF-7 cell line than to TK-10 and UACC-62. In contrast, compound **6** was less selective and presented cytotoxic activity on the three cells lines.

Table 1
Effects of arylfurans on the growth of human cancer cells lines and proliferation of human lymphocytes

Compounds[a]	Percent inhibition			Percent proliferation human lymphocytes
	MCF-7 (breast cancer)	TK-10 (renal cancer)	UACC-62 (melanoma)	
4	29 ± 4	17 ± 1	22 ± 2	83 ± 15
5	21 ± 11	14 ± 4	28 ± 1	91 ± 15
6	75 ± 12	89 ± 44	110 ± 21	72 ± 13[b]
7	43 ± 8	22 ± 9	30 ± 8	75 ± 8[b]
8	23 ± 4	16 ± 8	13 ± 2	106 ± 9
9	31 ± 6	10 ± 4	18 ± 0.2	93 ± 22
10	90 ± 3	19 ± 3	44 ± 11	113 ± 53
11	19 ± 2	30 ± 8	17 ± 4	87 ± 16
12	81 ± 9	33 ± 1	31 ± 18	72 ± 19[b]
13	73 ± 13	45 ± 9	50 ± 17	89 ± 9
14	19 ± 6	8 ± 1	37 ± 6	77 ± 28[b]
15	65 ± 17	17 ± 1	14 ± 8	94 ± 29
16	26 ± 10	11 ± 3	16 ± 7	89 ± 14
Etoposide	81 ± 11	41 ± 16	86 ± 15	–
Dexamethasone	–	–	–	58 ± 8

[a] Compounds **4**–**16** were tested at 20 μg/ml (50–120 μmol/l), etoposide was tested at 8 μg/ml (13.6 μmol/l) and dexamethasone was tested at 10 μg/ml (25.4 μmol/l).
[b] Compounds that inhibits the proliferation when compared with the control without drug ($P < 0.05$).

The ratios between $IC_{50(PBMC)}/GI_{50(tumor\ cell)}$ are also shown for some compounds in Table 2. This ration provides an indication of the selectivity of these compounds towards tumor cells when compared to normal cells, which were also tested in a high proliferative state after stimulation with PHA. Compounds **6**, **10** and **12** are poor inhibitors of the proliferation of PBMC as their IC_{50} were all above 300 μmol/l. The compound **10**, presenting an azo functionality, is much more active against MCF-7 (GI_{50} 1 ± 0.1 μmol/l) than compounds **6** and **12** (GI_{50} 54 ± 21 μmol/l). Interestingly, this increase in potency from **6** and **12** to **10** occurred without a corresponding increase in toxicity towards the activated PBMCs as the ratio IC_{50}/GI_{50} for **10** is higher than 300. The azo group is present in many bioactive drugs and its influence on selectivity may be due to a) bioisosterism with amide, heterocycles and double bond, b) a "spacer" effect, and c) free radical generation [16,17].

5. Conclusion

The compounds **6**, **10** and **12** were more active and exhibited better selectivity towards cancer cells than lignans **1**–**3**, used as prototypes. Further studies are necessary to assess the real potential of these compounds or their derivatives as drug leads. Work in this direction is in progress.

6. Experimental

6.1. Chemistry

All melting points were determined on Electrothermal IA9000 series digital melting point apparatus and are uncorrected. The NMR spectra were measured in $CDCl_3$ or [d_6]-DMSO with TMS as the internal standard with a Bruker *Avance* 200 instruments. Chemical shifts are given in δ scale and J values are given in Hz. Column chromatography was performed with silica gel 60, 70–230 mesh (Merck). All starting materials were commercially available research-grade chemicals and used without further purification. We previously reported the structural characterization of arylfurans **4**–**8**, **12**, **13** and **15** [9].

6.1.1. General procedure for the Meerwein arylation

A solution of substituted anilines (5 mmol) in water (10 ml) and concentrated HCl (3 ml) was cooled to 5 °C and diazotized with a solution of $NaNO_2$ (5.1 mmol) in H_2O (5 ml). After 10 min, a solution of 2.5 mmol of furan in acetone (20 ml) and 0.75 mmol of $CuCl_2{\cdot}2H_2O$ were added to the stirred reaction mixture. The reaction mixture was left to stand at room temperature for 24 hours. After dilution with water the precipitated crystals were filtered off, washed with abundant water and purified by column chromatography.

*6.1.1.1. 2,5-Bis-(4-chlorophenyl)furan (**9**)*. The arylfuran **9** was prepared from 4-chloroaniline using the method described above. Yield: 6%; pale yellow crystals; m.p. 166.4–167.3 °C (petroleum ether–diethyl ether) (Ref. [18]: 169–170 °C); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 6.70 (2H, s, 2 × H-furan); 7.35 (4H, d, J = 8.6 Hz, 4 × H-arom.); 7.63 (4H, d, J = 8.6 Hz, 4 × H-arom.).

*6.1.1.2. Azofuran (**10**)*. The arylfuran **10** was prepared from 4-anisidine using the method described above. Yield: 2%; red solid; m.p. 111.2–112.4 °C (hexane–AcOEt); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.85 (3H, s, $OC\underline{H}_3$); 3.88 (3H, s, $OC\underline{H}_3$); 6.79 (1H, d, J = 3.7 Hz, H-furan); 6.95 (2H, d, J = 9.0 Hz, 2 × H-arom.); 6.99 (2H, d, J = 9,0 Hz, 2 × H-arom.); 7.05 (1H, d, J = 3.7 Hz, H-furan); 7.78 (2H, d,

Table 2
GI_{50} for human cancer cell lines and IC_{50} for human PBMC stimulated with PHA for the most potent compounds

Compounds	GI_{50} (μmol/l)			IC_{50} (μmol/l)
	MCF-7 (breast cancer)	TK-10 (renal cancer)	UACC-62 (melanoma)	Human lymphocytes
6	54 ± 21 (> *5.6*)[a]	30 ± 11 (> *10*)[a]	22 ± 6 (> *13.6*)[a]	> 300
10	1.0 ± 0.1 (> *300*)[a]	> 100 –	51 ± 15 (> *5.9*)[a]	> 300
12	49 ± 4 (> *10.2*)[a]	> 100 –	> 100 –	> 500
Etoposide	0.03 ± 0.01	20.4 ± 2.3	1.4 ± 0.4	–
Dexamethasone	–	–	–	31 ± 1.7

The results are expressed as concentration (μmol/l) ± S.D. for three independent experiments run in triplicates.
[a] $IC_{50(PBMC)}/GI_{50(tumoral\ cells)}$.

$J = 9.0$ Hz, 2 × H-arom.); 7.88 (2H, d, $J = 9.0$ Hz, 2 × H-arom.); high-resolution ms calcd for $C_{18}H_{16}N_2O_3$: 308.11609. Found: 308.11507.

*6.1.1.3. 2-(4-Carboxyphenyl)furan (**11**)*. The arylfuran **11** was prepared from 4-aminobenzoic acid using the method described above. Yield: 19%; white solid; m.p. 228.1–229.0 °C (hexane–AcOEt) (Ref. [19]: 226 °C); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 6.63 (1H, dd, $J = 3.4$ and 1.8 Hz, H-furan); 7.12 (1H, d, $J = 3.4$ Hz, H-furan); 7.78–7.83 (3H, m, H-furan and 2 × H-arom.); 7.98 (2H, d, $J = 8.5$ Hz, 2 × H-arom.).

*6.1.1.4. 3,4-Dicarboxyethyl-2-(4-sulfonamidephenyl)furan (**14**)*. The arylfuran **14** was prepared from sulfanilamide and diethyl 3,4-furandicarboxylate using the method described above. Yield: 39%; white solid; m.p. 137.4–137.8 °C (hexane–CH_2Cl_2); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.27 (6H, t, $J = 6.9$ Hz, 2 × $C\underline{H}_3CH_2O$); 4.4–4.21 (4H, m, 2 × $CH_3C\underline{H}_2O$); 7.49 (2H, sl, $N\underline{H}_2$); 7.82 (2H, d, $J = 8.3$ Hz, 2 × H-arom.); 7.94 (2H, d, $J = 8.3$ Hz, 2 × H-arom.); 8.59 (1H, s, H-furan); EM: *m/z* 367, 322, 294, 213, 184, 60, 44, 28.

*6.1.1.5. 4-(2-Furyl)-N-(6-methoxy-3-pyridazinyl)-benzenosulfonamide (**16**)*. The arylfuran **16** was prepared from sulfamethoxypiridazine using the method described above. Yield: 36%; white solid; m.p. 164–165 °C (hexane–AcOEt); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.82 (3H, s, $OC\underline{H}_3$); 6.63 (1H, dd, $J = 3.3$ and 1.7 Hz, H-furan); 7.10 (1H, d, $J = 3.3$ Hz, H-furan); 7.37 (1H, d, $J = 9{,}8$ Hz, H-pyridazinyl); 7.7–7.9 (7H, m, H-furan, $N\underline{H}$, 4 × H-arom., H-pyridazinyl); high-resolution ms calcd for $C_{15}H_{13}N_3O_4S$: 331.06268. Found: 331.06207.

6.2. Assay with tumor cells

The effect of arylfurans on the growth of tumor cell lines was evaluated according to the procedure adopted by the National Cancer Institute for the in vitro anticancer drug screening that uses the protein-binding dye SRB to estimate cell growth. The methodology used was the same as originally published by the NCI team [12]. Three human tumor cell lines were used, MCF-7 (breast cancer), TK-10 (renal cancer), and UACC-62 (melanoma). All cells were cultured in RPMI medium supplemented with 5% FCS and gentamicin. Shortly before reaching confluence the cells were detached with trypsin-EDTA and seeded into 96-well plates so that 100 μl per well contained 10,000 UACC-62 and MCF-7 cells, and 15,000 TK-10 cells. After 24 hours of incubation, 80 μl of medium and 20 μl of the compound solutions (200 μg/ml in 2% aqueous DMSO) were added. After 48 hours in the presence of the compounds, the cells were fixed by adding 50 μl of cold 50% (w/v) trichloracetic acid (TCA) to each well and incubating the plate at 4 °C for 1 h. The supernatant was then discarded and the cells washed five times with water. After drying at room temperature, 50 μl of SRB solution (0.4% w/v in 1% acetic acid) was added to each well, and the plate incubated for 30 min at 4 °C. Unbound SRB was removed by washing five times with 1% acetic acid and the plates dried at room temperature overnight. The plates were read at 515 nm after dissolution of the dye with Tris buffer. Etoposide was used as positive controls and DMSO 0.2% as negative control. Each experiment was performed in triplicate. Representative data of mean and S.D. of at two independent experiments.

6.3. Lymphocyte assays

Assay with PBMC was run as previously, with modifications [13]. Shortly, PBMC of health adults were separated by Ficoll gradient. PBMC (1.5×10^5 cells per well) were cultured in RPMI-1640 medium, supplemented with 5% (v/v) heat-inactivated, pooled AB serum and 2 mM L-glutamine and antibiotic/antimicotic solution containing 1000 U/ml penicillin, 1000 μg/ml streptomycin and 25 μg/ml fungisone in flat bottomed microtiter plates. The cultures were stimulated with 2.5 μg/ml of phytohemaglutinin (PHA) in presence of different compounds at a final concentration of 20 μg/ml and incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2. Proliferation was quantified using MTT [14]. Dexamethasone was used as positive controls in this assay. PBMC assay were performed in quadruplicate.

6.4. Statistical analysis

Data were analyzed with the Student's *t*-test when appropriate or by one-way analysis of variance and Bonferroni's multiple comparison test. P values < 0.05 were taken to be significance.

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