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Bioorganic & Medicinal Chemistry Letters 17 (2007) 1799–1802
Bioorganic & Medicinal Chemistry Letters

Synthesis of novel diaryl ethers and their evaluation as antimitotic agents

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Received 21 September 2006; revised 6 November 2006; accepted 13 December 2006
Available online 21 December 2006

Abstract—A series of novel diaryl ethers possessing various functional groups were synthesized and evaluated for antiproliferative activity in human myeloid leukemia HL-60 cells. Among the compounds examined, compounds **10**, **17**, **20**, **24**, and **33** showed moderate to potent antiproliferative activity. These derivatives were further examined in terms of their abilities to inhibit tubulin polymerization; however, all of the tested compounds were relatively ineffective. The reference compound E7010 with an IC_{50} of 0.34 μM exhibited potent antiproliferative activity and significantly inhibited tubulin polymerization in a dose-dependent manner.

Antimitotic agents, which arrest cells in mitosis (the M phase of the cell cycle), can be classified as tubulin interactive agents (TIAs).[1] These tubulin-targeting agents are generally divided into two major classes: microtubule-stabilizing agents, like taxanes, epothilones, and discodermolide, and microtubule-destabilizing agents, like colchicine, vinca alkaloids, and cryptophycins.[2,3] Although taxanes and vinca alkaloids are widely used clinically to treat cancer, their structural complexities, difficult formulations, lack of oral availability, and more importantly, acquired and intrinsic resistance render these drugs suboptimum for clinical treatment of cancer.[4] Consequently, considerable interest is being shown in the discovery and development of novel small molecule inhibitors of tubulin polymerization that can circumvent the difficulties of natural products. In this context, progress has recently been made on the development of highly potent tubulin inhibitors and currently, several compounds are undergoing clinical trials.[5–10]

Diaryl ethers represent an important class of synthetic compounds recognized as potential anticancer drugs. Experimental and pre-clinical models have demonstrated that a number of these compounds elicit outstanding anticancer activity through the significant inhibition of tubulin assembly accompanied by potent antiproliferative activity.[11,12] Moreover, the diaryl ether scaffold is found in a number of natural products and biologically important molecules.[13] In this context, several reports have recently described various pharmacological evaluations of compounds possessing diaryl ether motifs.[14–16] As a result of our continuing studies aimed at the discovery and development of potential antimitotic agents, herein we report the synthesis, antiproliferative activities, and tubulin polymerization inhibitions of a series of novel diaryl ethers.

A variety of substituted diaryl ethers, **3**–**25** and **28**–**33**, were prepared as outlined in Schemes 1–3. To facilitate the efficient and diverse synthesis of the key diaryl ether skeleton,[17] the approach that emerged as being most attractive was the $Cu(OAc)_2$-mediated coupling reaction developed by the Evans group,[18] due to its mild reaction conditions as compared with alternatives[17] (room temperature versus higher temperature, >80 °C). Thus, treatment of 4- or 3,4-substituted arylboronic acids **1** and appropriate phenols **2** with $Cu(OAc)_2$ in the

Keywords: Antimitotic agents; Tubulin polymerization; Diaryl ether; Antiproliferative activity.
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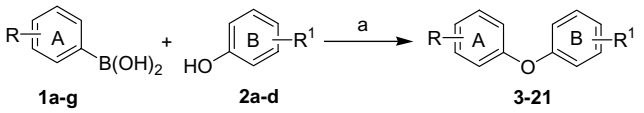
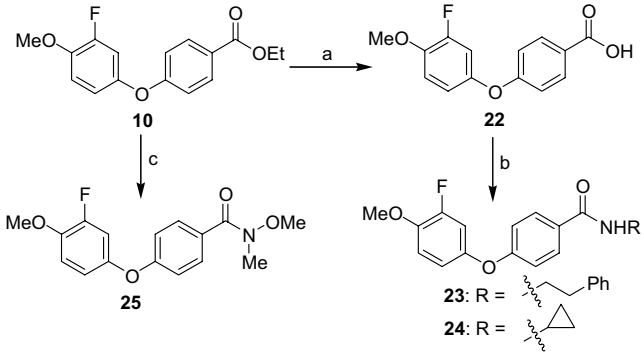
doi:10.1016/j.bmcl.2006.12.048



R = 4-CN, 4-Cl, 4-NMe_2, 3-F,4-OMe, 4-OMe, 3,4-di-OMe, 3,4-di-F.
R^1 = 3-CO_2Me, 4-COOEt, 3-OH-4-COOEt, 2,5-di-OMe.

Scheme 1. Reagents and condition: (a) $Cu(OAc)_2$, Et_3N, CH_2Cl_2, rt 42–84%.

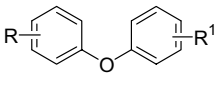


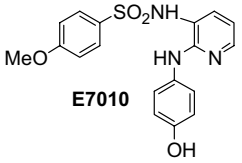
Scheme 2. Reagents: (a) LiOH, H_2O–THF, 86%; (b) phenethylamine, DCC, DMAP, THF, 53% for **23**; cyclopropylamme, DCC, DMAP, THF, 77% for **24**; (c) Me(MeO)NH · HCl, *i*-PrMgCl, THF, 63%.

presence of Et_3N and molecular sieve at room temperature afforded the desired diaryl ethers **3**–**21**[19] in modest to high yields (Scheme 1).[20]

As shown in Scheme 2, amide derivatives **23**–**25**[19] were also prepared starting from ester **10**, which appeared to have promising antiproliferative activity (*vide infra*, Table 1). To this end, ester **10** was subjected to alkaline hydrolysis to give the corresponding acid **22**. DCC-mediated coupling of the resulting **22** with appropriate amines provided the amide analogues **23** and **24**, in 53% and 77% yields, respectively. Reaction of ester **10** with *N*,*O*-dimethylhydroxylamine in the presence of isopropyl magnesium chloride[21] provided the *N*-methoxy-*N*-methylamide derivative **25**.

Table 1. In vitro antiproliferative activities of diaryl ethers **3**–**21** and E7010 in HL-60 cells[a]

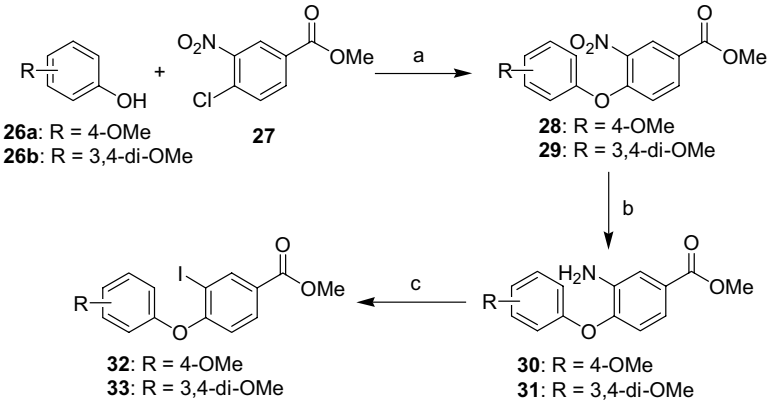




Compound	R	R^1	IC_{50}[a] (μM)
3	4-CN	3-COOMe	>20
4	4-$N(Me)_2$	3-COOMe	>20
5	4-OMe	3-COOMe	>20
6	3-F,4-OMe	3-COOMe	>20
7	4-CN	4-COOEt	>20
8	4-$N(Me)_2$	4-COOEt	>20
9	4-OMe	4-COOEt	>20
10	3-F,4-OMe	4-COOEt	3.5
11	4-Cl	4-COOEt	>20
12	3,4-Di-OMe	4-COOEt	>20
13	3,4-Di-F	4-COOEt	>30
14	4-$N(Me)_2$	3-OH, 4-COOEt	>30
15	4-OMe	3-OH, 4-COOEt	>30
16	3-F,4-OMe	3-OH, 4-COOEt	>30
17	3,4-Di-OMe	3-OH, 4-COOEt	1.5
18	3,4-Di-F	3-OH, 4-COOEt	>30
19	4-CN	2,6-Di-OMe	>20
20	4-$N(Me)_2$	2,6-Di-OMe	11.4
21	3-F,4-OMe	2,6-Di-OMe	>20
E7010			0.34

[a] All experiments were independently performed at least three times.

To identify the effect of *ortho*-substitution of the B-ring on antiproliferative activity, *ortho*-substituted diaryl ethers **28**–**33** were obtained by utilizing a sequence of reactions (Scheme 3). Ullmann coupling[17] of the potassium salts of appropriate phenols **26** with methyl 4-chloro-3-nitrobenzoate (**27**) gave the corresponding diaryl ethers **28** and **29** possessing an *ortho*-nitro group, which were subsequently reduced to the related aniline derivatives **30** and **31**. Diazotization followed by iodination of these anilines, **30** and **31**, gave the respective iodinated diaryl ethers **32** and **33**[19] in good yields.



Scheme 3. Reagents and conditions: (a) aq KOH, reflux, 76–88%; (b) Pd/C, H_2AcOH, rt; (c) (i) $NaNO_2$, concd H_2SO_4, 0–5 °C; (ii) KI, H_2O, rt-70 °C, 55–71% for two steps.

Newly synthesized diaryl ethers **3**–**25** and **28**–**33** were initially screened for their in vitro antiproliferative activity against human myeloid leukemia HL-60 cells. Results are tabulated as IC_{50} values in Tables 1–3.

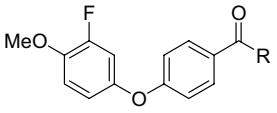
E7010, a novel sulfonamide antimitotic agent, was used as a reference drug and as expected, this compound displayed potent antiproliferative activity with an IC_{50} of 0.34 μM.[22,23] All assays were performed under standard assay conditions by following a previously described assay protocol.[23] The in vitro inhibition data for compounds **3**–**21** which were obtained using a straightforward single-step reaction are presented in Table 1.[23] Initially, we explored the effects of various activating groups on ring A whilst maintaining the ester moiety at positions 3 or 4 on ring B; analogues **3**–**13**. Of these, analogue **10** with 3-fluoro-4-methoxy groups on A-ring exhibited significant antiproliferative activity with an IC_{50} of 3.5 μM, whereas all of the other derivatives displayed poor inhibitory activity. In view of the potent activity of **10**, we prepared more analogues **14**–**18** by introducing a hydroxy group at position 3 of ring B. This modification resulted in significant inhibition enhancement, as represented by analogue **17**, which had an IC_{50} of 1.5 μM. On the other hand, the other compounds showed significantly poor inhibition. Interestingly, compound **16**, the 3-hydroxy derivative of **10**, displayed poor inhibitory activity. Analogue **20** with methoxy groups at the 2 and 6 positions of ring B also exhibited considerable activity with an IC_{50} of 14.1 μM. Based on the potent inhibitory activities of diaryl ethers **10** and **17**, we investigated the effects of replacing ester moieties with acid and amide groups.

As shown in Table 2, these derivatives showed markedly reduced potency with the exception of amide **24** which had an IC_{50} of 14.1 μM. This result suggested that the size of the R (the ethoxy group of **10** and the cyclopropylamide group of **24**) would be important for potency. To evaluate the significance of *ortho*-substitution on the B-ring, we synthesized compounds **28**–**33**. Of these compounds, the iodo-derivative **33** exhibited considerable potency with an IC_{50} of 5.62 μM, whereas, the nitro- and amino-analogues showed significantly poor inhibition (Table 3). However, all of the active compounds were less potent than the reference compound E7010. Although much is not evident from these preliminary structure–activity relationship studies, these findings offer new possibilities for further improving potency.

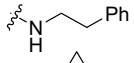
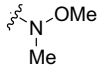
To investigate whether these diaryl ethers affect microtubule assembly, compounds **10**, **17**, **20**, **24**, and **33**, which exhibited moderate to potent antiproliferative activity in HL-60 cells, were further tested with respect to tubulin polymerization inhibition.[24] E7010 was used as a positive control. Inhibitory activity of the tested compounds was determined using HTS-Tubulin Polymerization Assay Kits (Cytoskeleton, CDS01) and a previously reported assay protocol.[24] The results shown in Table 4 are presented as percentage inhibitions at the tested concentrations. E7010 inhibited tubulin polymerization in a dose-dependent manner, and complete inhibition was observed at 10 μg/mL. On the other hand, compounds **10**, **17**, **20**, and **24** showed poor inhibitory profiles ranging from 13.3% to 8.7% inhibition, and compound **33** was inactive at 10 μM.

In summary, a series of novel diaryl ethers possessing various functional groups were synthesized and initial antiproliferative activity screening using human myeloid leukemia HL-60 cells showed that some of these

Table 2. In vitro antiproliferative activity of diaryl ethers **22**–**25** in HL-60 cells[a]

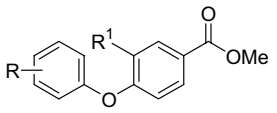


22-25

Compound	R	IC_{50}[a] (μM)
22	OH	>30
23		20.6
24		14.1
25		>30

[a] All experiments were independently performed at least three times.

Table 3. In vitro antiproliferative activity of diaryl ethers **28**–**33** in HL-60 cells[a]



28-33

Compound	R	R^1	IC_{50}[a] (μM)
28	4-OMe	NO_2	>20
29	3,4-Di-OMe	NO_2	>20
30	4-OMe	NH_2	>20
31	3,4-Di-OMe	NH_2	>20
32	4-OMe	I	>20
33	3,4-Di-OMe	I	5.62

[a] All experiments were independently performed at least three times.

Table 4. Inhibition of tubulin polymerization by diaryl ethers **10**, **17**, **20**, **24**, **33**, and E7010[a]

Compound	Concentration (μg/mL)	% of inhibition[a]
Control	—	0%
10	10	12.5%
17	10	5.5%
	20	13.3%
20	10	12.5%
24	10	8.7%
33	10	Inactive
E7010	1	40 %
	3	74.6%
	10	96.3%

[a] All experiments were independently performed at least two times.

analogues, that is, **10**, **17**, **20**, **24**, and **33**, had potent to moderate inhibitory activities. Based on these results, further evaluations were conducted to investigate the abilities of theses analogues to inhibit tubulin polymerization. However, all of the tested compounds exhibited poor inhibition. Further study for mode of action of these aryl ethers is under progress.

Taken together, our findings provide important information of the structural features that influence the functional activities of this class of compounds, and offer new possibilities for further explorations to improve potency.

Acknowledgments

This research was supported by the Regional Research Centers Program of the Ministry of Education & Human Resources Development and by a grant of the Korean Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ2-PG6-DC02-0001).

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19. All new synthetic compounds gave satisfactory analytical and spectral data. Selected spectral data for compound **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, J = 8.9 Hz, 2H), 6.88 (d, J = 8.9 Hz, 3H), 6.71–6.92 (m, 3H), 4.29 (q, J = 7.1 Hz, 2H), 3.83 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0, 161.7, 153.6, 151.6, 148.7, 144.8, 131.6, 124.9, 116.7, 115.6, 114.2, 109.4, 60.8, 56.7, 14.3; IR (thin film, neat) 1715 cm^{-1}; MS (FAB$^+$) *m/z* 290 (M$^+$). Compound **17**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.0 (s, 1H), 7.79 (d, J = 8.9 Hz, 1H), 6.90 (d, J = 9.4 Hz, 2H), 6.56 (s, 1H), 6.50 (dd, J = 8.8 Hz, 2.34, 1H), 6.42 (s, 1H), 4.39 (q, J = 7.2 Hz, 2H), 3.9 (s, 3H), 3.85 (s, 3H), 1.4 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 164.8, 163.6, 150.0, 148.4, 146.4, 131.5, 112.2, 111.8, 108.6, 107.0, 105.3, 104.2, 61.2, 56.3, 56.0, 14.2; IR (thin film, neat) 1668 cm^{-1}; MS (FAB$^+$) *m/z* 318 (M$^+$).
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24. Inhibition of tubulin polymerization. Tubulin assembly was measured turbidimetrically using a Molecular Device SPECTRA MAX plus model equipped with electronic temperature controllers. HTS-tubulin Polymerization Assay Kit (Cytoskeleton, CDS01) was used to test tubulin assembly. Test compounds were dissolved in DMSO to the desired concentrations. All components were added to microwells held at 0 °C, and baselines were established at 340 nm. Assembly was measured sequentially at 0 and 37 °C. See; Kuo, C. C.; Hsieh, H. P.; Pan, W. Y.; Chen, C. P.; Lion, J. P.; Lee, S. J.; Chang, Y. L.; Chen, L. T.; Chen, C. T.; Chang, J. Y. *Cancer Res.* **2004**, *64*, 4621.