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Copper salt-catalyzed formation of a novel series of triazole–spirodienone conjugates with potent anticancer activity†

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Copper salt-catalyzed oxidative amination resulted in the formation of a novel series of triazole–spirodienone conjugates, 4-triazolyl-1-oxa-4-azaspiro[4,5]deca-6,9-dien-3,8-diones and 4-triazolyl-1-oxa-4-azaspiro[4,5]deca-6,9-dien-8-ones. A single crystal of compound **1p** among them was grown and analyzed by X-ray crystallography. These compounds were evaluated for their antiproliferative activities against MDA-MB-231, HeLa, A549 and MCF-7 cell lines. Most of them showed moderate to high anticancer potency in the four cancer cell lines. The discovery of the triazole–spirodienone conjugates as cytotoxic agents against cancer cells may open up a new field in which these novel small molecules could be further explored as promising anticancer agents.

Introduction

More than one hundred drugs have been approved by the United States Food and Drug Administration (USFDA) for clinical treatment of cancers over the last half century, yet the search for new chemical entities as potential anticancer agents continues in the hope that improved pharmaceutical profiles may come from hitherto unknown molecular structures that encode the keys to greater efficacy and more manageable adverse side effects. Among numerous drug discovery approaches such as high-throughput screening, molecular modeling, and natural compound mimetics, one method may prove effective that takes pharmacophores important in existing anticancer agents on which to rationally design modified structures that may confer a better therapeutic index.

Quinone is a common moiety of numerous biologically active molecules, including natural and synthetic anticancer drugs such as doxorubicin,[1] mitoxantrone,[2] andaunorubicin[3] and mitomycin C.[4] The role of the quinone moiety

appears to be important in endowing bioactivities in the molecules ranging from simple small quinones to large complex quinoid systems.[5–9] Thus the utility of quinone-containing structures has been extensively explored in search of anticancer drug candidates, leading to an ever increasing library of new promising quinones with anticancer activities.[10–16] We have previously obtained some quinone derivatives and conjugates as anticancer agents.[17,18] In another previous work on quinones,[19] we found that *N*-sulfonamide spirodienone derivatives (Fig. 1) demonstrated moderate cytotoxic activities against HCT-8, Bel-7402, BGC-823, A549 and A2780 cell lines with IC_{50} values in the range of 1.93–51 μM.

The introduction of a 1,2,4-trizole[20,21] ring into molecules has been reported to significantly increase anticancer activity or improve the bioavailability and overall pharmacological profile of a drug candidate.[22–26] Indeed, clinically proven anticancer drugs[27,28] such as letrozole, vorozole, and anastrozole all contain a 1,2,4-triazole nucleus which is stable to metabolism and acts as an important pharmacophore at the active site of receptors as hydrogen bond acceptor or donor. We hypothesize that conjugating quinones with a 1,2,4-triazole nucleus may generate novel molecular entities with desirable anticancer activities. Therefore, *N*-sulfonamide-spiro-dienones were chosen as the lead compound and triazole–spirodienone conjugates were designed by replacing the sulfonyl functional group with a 1,2,4 (the relative position of ring nitrogen)-triazole nucleus (Fig. 1). Herein, we report the synthesis and anticancer evaluation of the designed triazole–spirodienone conjugates: 4-triazolyl-1-oxa-4-azaspiro[4,5]deca-6,9-dien-3,8-diones (**1**) and 4-triazolyl-1-oxa-4-azaspiro[4,5]-deca-6,9-dien-8-ones (**2**).

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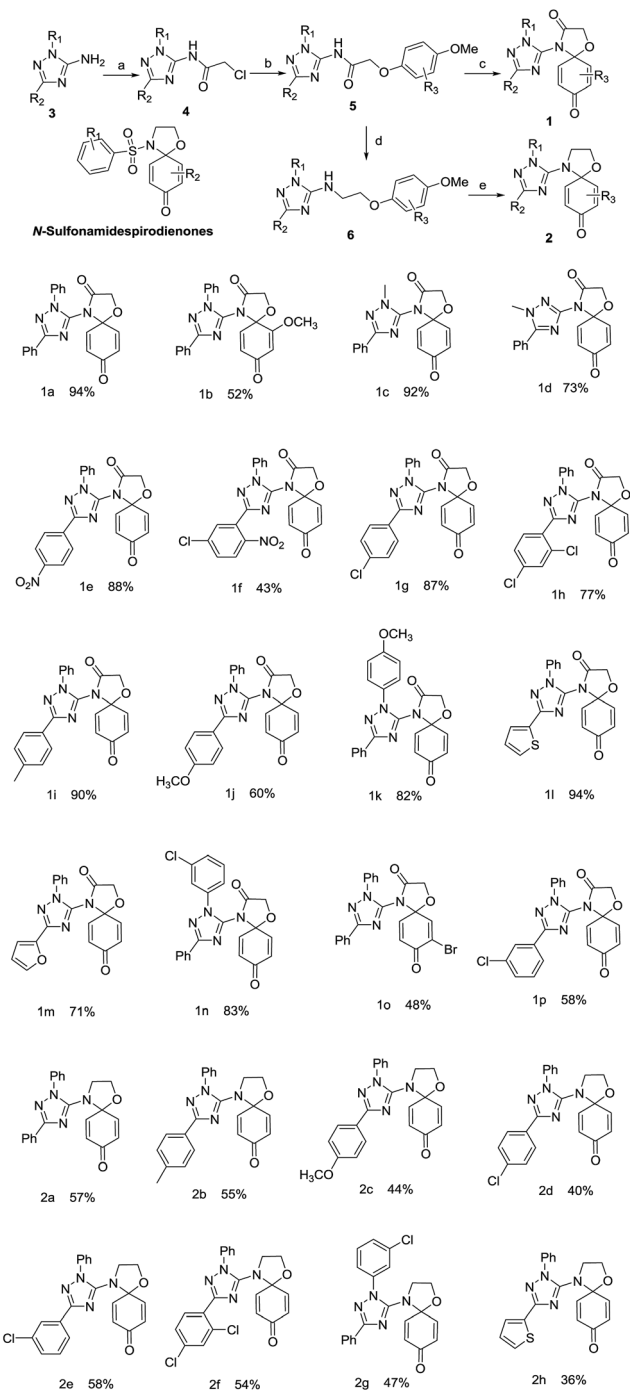
Fig. 1 N-Sulfonamide spirodienones and synthetic scheme of designed novel triazole−spirodienone conjugates (**1** and **2**). Reagents and conditions: (a) chloroacetyl chloride, (Et)₃N, CH₂Cl₂; (b) R₃-PhONa, DMF; (c) [Cu(CH₃CN)₄]ClO₄, PhI(CF₃CO₂)₂, CH₂Cl₂, rt; (d) LiAlH₄, AlCl₃; (e) Cu(CF₃SO₂)₂, PhI(CF₃CO₂)₂, CH₂Cl₂, rt.

Results and discussion

Chemical synthesis

The target conjugates were prepared following the synthetic scheme as depicted in Fig. 1. The starting materials 1,2,4-triazoles (**3**) were prepared in an efficient one-pot procedure

including the cyanoimidation of aldehydes and cyclization.[29] Following acylation of **3** to form 1,3-substituted-2-chloro-N-(1H-1,2,4-trizol-5-yl)acetamides (**4**), the sodium phenoates attacked **4** to provide N-(1,3-disubstituent-1H-1,2,4-triazol-5-yl)-2-phenoxyacetamides (**5**). The reduction of **5** gave 1,3-dis-ubstituent-N-(2-phenoxyethyl)-1H-1,2,4-triazol-5-amines (**6**). Subsequently, the key step is the oxidative amination reac-tion[19,30,31] of **5** and **6** leading to their corresponding 4-triazolyl-1-oxa-4-azaspiro[4,5]deca-6,9-dien-3,8-dione (**1**) and 4-triazolyl-1-oxa-4-azaspiro[4,5]deca-6,9-dien-8-one (**2**) in moderate to high yields.

The formations reaction of **1a** and **2a** were taken as an example for optimization of reaction conditions of the oxidative amination using the previous work as ref. 19 and 31 The results (Table 1) show that copper(II), rhodium(II) and ruthenium(II) could be used as a catalyst for the amination of aromatic ether **5** and **6**. Other metal complexes, such as those of Mn(II), Co(II) and Fe(II) dis-played poor catalytic activities. At same time, we found that the yields increased inconspicuously when the loading of [Cu(CH₃-CN)₄]ClO₄ and Cu(CF₃SO₂)₂ increased from 5% to 20%. In addi-tion, PhI(OAc)₂ and PhI(CF₃CO₂)₂ were employed effectively as oxidant for the oxidative amination of amides (**5**) and amines (**6**). Finally, the optimized conditions for the formation of triazole–spirodienones **1a** and **2a** are in the molar ratio of 1 : 2.5 : 2.5 : 0.1

Table 1 Effect of catalysts and oxidants on the amination[a]

| Entry | Catalyst | Oxidant | Yield[b] (%) | |
			1a	**2a**
1	—	PhI(CF₃CO₂)₂	—	—
2	10% CuCl₂	PhI(CF₃CO₂)₂	32	Trace
3	10% CuBr₂	PhI(CF₃CO₂)₂	32	Trace
4	10% Cu(CF₃SO₃)₂	PhI(CF₃CO₂)₂	**49**	**57**
5	10% Cu(acac)₂	PhI(CF₃CO₂)₂	36	28
6	10% Cu(OAC)₂	PhI(CF₃CO₂)₂	30	22
7	10% Cu(CF₃COCH₂COCF₃)₂	PhI(CF₃CO₂)₂	36	29
8	10% Cu[(CH₃CN)₄]ClO₄	PhI(CF₃CO₂)₂	**94**	**40**
9	5% Rh₂(OAc)₄	PhI(CF₃CO₂)₂	33	Trace
10	5% Ru(TTP)CO	PhI(CF₃CO₂)₂	44	Trace
11	10% Cu(CF₃SO₃)₂	PhI(OAc)₂	35	**51**
12	10% Cu(CF₃SO₃)₂	PhI=O	27	Trace
13	10% Cu[(CH₃CN)₄]ClO₄	PhI(OAc)₂	45	**47**
14	10% Cu[(CH₃CN)₄]ClO₄	PhI=O	30	Trace

[a] Substrate(1 mmol), oxidant (2.5 mmol), Al₂O₃(2.5 mmol); CH₂Cl₂ as solvent, room temperature, 4 Å molecular sieve. [b] Isolated yield based on the amount of compounds **1a** and **2a** consumed.
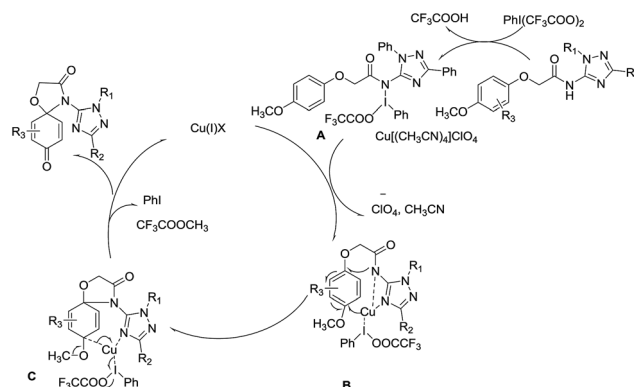
of substrate : $PhI(CF_3CO_2)_2$: Al_2O_3 : $Cu[(CH_3CN)_4]ClO_4$ for **1a** or $(Cu(CF_3SO_2)_2)$ for **2a** at room temperature. Next, the reaction conditions of **1a** and **2a** were expanded to the formation reactions of all triazole–spirodienones **1** and **2**, respectively. Thus, twenty four triazole–spirodienone **1** and **2** were obtained in 36–94% yield (Fig. 1) from corresponding 2-phenoxy-ethyl-acet-*N*-2,3,5-triazoleamides and 2-phenoxy-ethyl-ethyl-*N*-2,3,5-triazoleamines.

In entry, the effects of the substituents on the oxidative amidation reaction were noticed that the electron-donating groups sped the oxidative amination reaction of the aromatic ether derivatives (**5** and **6**) and the electron-withdrawing substituents resulted in moderate yields of the reaction, thereby, the reaction rates rely on the easy of oxidation of the substrate and the yields of triazole–spirodienone (**1** and **2**) are linked with the activity of the nitrogen sources, especially, the effect of N–H dissociation energies of the nitrogen source.

To confirm the structures of triazole spirodienone conjugates, a single crystal of **1p** was grown and analyzed by X-ray crystallography (the detailed crystal data are provided in the ESI†). The ORTEP structure plot of **1p** is displayed in Fig. 2.

The effect of substituent was investigated using different substituted aromatic ether derivatives as substrates under strictly identical condition. Interestingly, we notice that the reaction of these methoxy group substituent in the *meta*-position gave only two corresponding six-membered heterocyclic products **3a** and **3b** in 53% and 24% yield respectively (Scheme 1).

A mechanism is proposed in Scheme 2 for the oxidative amination reaction of the aromatic ether with β-NH–imidazole based on the mechanisms proposed originally for the amination by Antonchick[32] and Chiba.[33] Initially ditrifluoro-acetoxyiodobenzene reacts with the amide to give intermediate A, which is then transformed into intermediate B through an oxidative insertion of N–I bond by copper(II). The reductive elimination of intermediate C releases copper(I) salt, trifluoro-acetic acid and iodobenzene, leading to final product 4-(1,3-diphenyl-1*H*-1,2,4-triazol-5-yl)-1-oxa-4-azaspiro[4.5]-deca-6,9-diene-3,8-dione (**1**). And the 4-(1,3-diphenyl-1*H*-1,2,4-triazol-5-

Scheme 2 Proposed mechanism for oxidative amination.

yl)-1-oxa-4-azaspiro[4.5]deca-6,9-diene-8-one (**2**) was obtained in similar mechanisms.

Antiproliferation activity against a panel of cancer cell lines

All of triazole–spirodienone conjugates (**1** and **2**) were examined for *in vitro* antiproliferation activities against a triple negative human breast cancer cell line, MDA-MB-231, an ER + human epithelial mammary carcinoma cell line, MCF-7, an invasive cervical cancer cell line, HeLa, and a metastatic non-small cell lung cancer cell line, A549. Table 2 displayed the IC_{50} values of **1** and **2** against these four cell lines. Moderate to high anti-proliferative activities were observed in most of **1** and **2** derivatives, with IC_{50} values ranging from low nanomolar concentration to micromolar concentration. The triazole–spirodienone conjugates inhibited cancer cell growth in a dose-dependent manner in all four cancer cell lines tested, and appear to exert a greater inhibitory effect on MDA-MB-231 cells in particular. Notably, 4-triazolyl-1-oxa-4-azaspiro[4,5]-deca-6,9-dien-3,8-diones (**1**) were generally stronger cell growth inhibitors than the corresponding 4-triazolyl-1-oxa-4-azaspiro[4,5] deca-6,9-dien-8-ones (**2**). The only structural difference between **1** and **2** is the extra carbonyl group on 3-position of the spirodienone ring of **1**, suggesting that introduction of a 3-one functional group at the spiro-ring increased the antiproliferation activity against cancer cells.

Cytotoxicity towards MCF-10A normal mammary epithelial cells

Seven of the most promising triazole–spirodienone conjugates (**1a**, **1d–f**, and **1i–k**) were selected for further evaluation of toxicity in normal mammary epithelial cells, MCF-10A. At 10 μM, all the compounds were found to be significantly toxic towards MCF-10A cells, with toxicities moderating at a lower dosage of 1 μM. At both doses of 1 and 10 μM, doxorubicin, a widely used anticancer drug in clinic, was found to be much more toxic to normal cells than any of the selected triazole–spirodienone conjugates (the detailed data see ESI†). This observation is encouraging because the novel structural motif of triazole–spirodienone conjugates may offer a better separation of desired tumor toxicity and toxicity to normal cells.
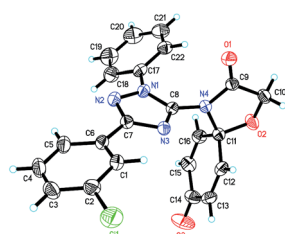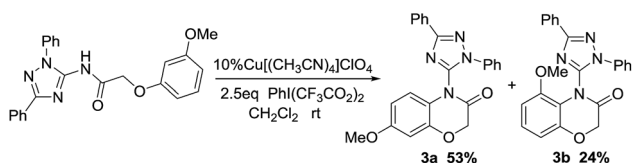

Fig. 2 ORTEP plot of molecular structure of **1p**.

Scheme 1 The reaction results of *m*-methoxy substituted aromatic ether derivatives as substrates.

Table 2 Antiproliferation activity against cancer cell lines of **1** and **2**

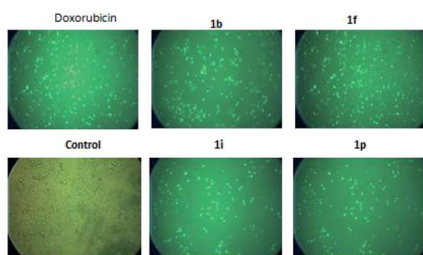| Compds | IC$_{50}$ (nM) | | | |
	MDA-MB-231	HeLa	A549	MCF-7
1a	20.3	93.9	288	40.7
1b	16.8	12.2	69.0	231
1c	17.1	95.5	395	111
1d	4.56	21.0	4.18	62.1
1e	67.8	326	1800	31.4
1f	6.44	17.5	457	16.2
1g	73.7	123	485	315
1h	270	308	399	397
1i	1.37	62.9	203	43.3
1j	22.2	25.2	266	116
1k	27.7	10.0	268	88.8
1l	113	727	346	245
1m	123	134	340	763
1n	157	107	484	1080
1o	453	381	31.1	465
1p	0.80	45.3	25.2	4.11
2a	470	123	357	694
2b	555	126	698	442
2c	60.6	976	465	843
2d	170	979	809	846
2e	309	753	1800	1530
2f	684	996	1010	476
2g	332	84.9	511	1100
2h	141	1240	1190	442

Fig. 3 Images of apoptosis induced by triazole–spirodienones conjugates, doxorubicin and vehicle control in HeLa cell lines.

Apoptosis of HeLa cells

We performed a caspase 3/7 activation assay for compounds **1b**, **1i**, **1f** and **1p** using fluorescence microscopy. HeLa cells were treated with four selected triazole–spirodienone conjugates at 1.0 μM or vehicle for 24 hours. In this assay, fluorescence signal was related to the degree of caspase 3/7 activation and hence apoptosis induced by the treatment. As shown in Fig. 3, untreated cells showed minimal fluorescence, while treated cells exhibited significant increase in fluorescence (shown in green) indicating the prevalence of apoptosis of HeLa cells. These observations strongly suggest that triazole–spirodienone conjugates inhibited HeLa cells proliferation by triggering apoptosis.

Conclusions

Copper salt-catalyzed oxidative amination resulted in the formation of novel triazole–spirodienone conjugates, 4-triazolyl-1-oxa-4-aza-spiro[4,5]deca-6,9-dien-3,8-diones (**1**) and 4-triazolyl-1-oxa-4-aza-spiro[4,5]deca-6,9-dien-8-ones (**2**) which were evaluated for their antiproliferative activities against MDA-MB-231, HeLa, A549 and MCF-7 cell lines. Most of them showed moderate to high anticancer potency in the four cancer cell lines. Moreover, 4-triazolyl-1-oxa-4-azaspiro[4,5]deca-6,9-dien-3,8-diones (**1**) were found to exhibit superior anticancer activities compared to 4-triazolyl-1-oxa-4-azaspiro[4,5]deca-6,9-dien-8-ones (**2**). The cytotoxicities of **1a**, **1d–f**, and **1i–k** toward non-cancerous cells (MCF-10A) were measurable, but much less severe than that of doxorubicin, a known and widely used chemotherapy agent. Our study represents the first report on the preparation and biological evaluation of the novel triazole–spirodienone conjugates as potential anticancer agents. The analogs (**1**) are promising lead compounds on which further optimization of structure–activity relationships may lead to therapeutically useful new chemical entities for clinical treatment of cancer. Preliminary study on the mode of action suggests that the triazole–spirodienone conjugates inhibited cancer cell proliferation by triggering apoptosis, with more in-depth mechanistic studies under way in our laboratories.

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