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Halogenated Diarylacetylenes Repress c-Myc Expression in Cancer Cells

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Abstract

Halogenated diarylacetylenes that possess fluorine or chlorine substituents in one aryl ring and N-methylamino or N,N-dimethylamino in the other aryl ring inhibit the proliferation of LS174T colon cancer cells through the repression of c-myc expression and induction of the cyclin-dependent kinase inhibitor-1 (*i.e.*, p21(Wif1/Cip1)) and represent potentially useful antineoplastic agents.

In the course of developing new agents for the treatment of colorectal cancer, we identified a family of fluorinated N,N- dialkylaminostilbene analogs (FIDAS agents) that inhibit the expression of Wnt target genes, such as c-myc[1], and repress colon cancer cell growth *in vitro* and *in vivo*[2–4]. Recently, we found that (*E*)-4-(2′,6′-difluorostyryl)-N,N-dimethylaniline (**1**) (Fig. 1) targeted exclusively the catalytic subunit[3] of methionine S-adenosyltransferase-2 (MAT-2) that serves as a source of S-adenosylmethionine (SAM) in colorectal and liver cancers where MAT-2 is upregulated[5–8]. Presumably, neoplastic tissues make effective use of SAM from this isoform of MAT to manage crucial epigenetic modifications of histone proteins and thereby regulate gene expression. Interference with this process represented a new approach for developing potential antineoplastic agents, and consequently, we explored related compounds that might alter c-myc expression. In

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addition, we sought compounds that would avoid the facile *E/Z*-isomerizations that afflict the stilbenes and that complicate pharmacodynamic and pharmacokinetic studies.

Among the possible analogs that would avoid this isomerization problem, the diarylacetylenes **2** (Fig. 1) were an obvious choice. The only prior reports of acetylenic compounds as antineoplastic agents include monoalkylacetylenes from aquatic organisms[9] and diarylacetylenic analogs of combrestatin[10]. The latter compounds showed cytotoxic activity against a murine leukemia cell line and one showed activity as an inhibitor of tubulin polymerization. The Sonogashira coupling[11,12] of 4-(N,N-dimethylamino) phenylacetylene with various aryl iodides provided access to the desired diarylacetylenes **2** (Table 1). Prior work from our laboratories established that stilbenes with N-methylamino and N,N-dimethylamino groups in a *para*-orientation relative to the central double bond as well as 2,6-difluoro, 2-chloro-6-fluoro or 2,6-dichloro halogenation patterns in the other aromatic ring were the most potent analogs in the repression of c-myc expression and colon cancer cell LS174T proliferation[2].

As a consequence, we limited SAR studies to those diarylacetylenes **2** that possessed fluorine or chlorine substituents in one aryl ring and N-methylamino or N,N-dimethylamino in the other aryl ring. We reported previously that stilbenes repressed colon cancer cell proliferation by inhibiting c-myc expression and inducing the cell cycle inhibitor, p21(Wif1/Cip1)[2]. The similarity of the diarylacetylenes to the stilbenes prompted an *in silico* modeling study of the binding of (*E*)-4-(2′,6′-difluorostyryl)-N,N-dimethylaniline (**1**) and 4-((2,6-difluorophenyl)ethynyl)-*N*,*N*-dimethylaniline (**2m**). Using a computationally constructed open-state model of the homodimer of MAT2A (see Supplementary Data for details), we observed that **1** and **2m** bound to the same active site (Fig. 2) and that the diarylacetylene **2m** inhibited MAT2 at concentrations comparable to that of the stilbene **1** (data not shown). Hydrophobic residues I139, F272, I274, and I344 define the boundaries of the binding pocket, and van der Waals contact between F272 and the halogen substituents accounted for the increased activity of *ortho*-halogens in **1** or **2m** over the corresponding analogs with *meta*- or *para*-halogens. In addition, a cleft formed by residues K203, S269, I274, and D280 accommodated the shape of these particular ligands (Fig. 2).

Variability in the MAT2A inhibition assay made the measurement of the levels of c-myc expression a preferred analytical tool for assessing the potency of diarylacetylenes. We tested the effect of these diarylacetylenes **2** on the proliferation of LS174T colon cancer cells. The expression of c-myc and p21(Wif1/Cip1) was analyzed by western blotting (Fig. 3). The most active diarylacetylenes **2** inhibited c-myc expression at 1 μM concentrations and as expected, induced p21(wif1/Cip1) at the same time. Consistent with prior results in the stilbene family, the diarylacetylenes **2** lacking halogen substituents (*e.g.*, **2c**) or possessing only one fluorine substituent at a *meta*-position relative to the acetylenic linkage (*e.g.*, **2f**) had very low potency (Table 1). Diarylacetylenes with one or two halogen substituents at *ortho*-positions relative to the acetylenic linkage (*e.g.*, **2b**, **2d**, **2e**, **2m** and **2n**) possessed potencies as inhibitors of LS174T cell proliferation that exceeded that of the related stilbene **1** with IC_{50} values less than 50 nM (Table 1). Isomers of these diarylacetylenes (*e.g.*, **2f**, **2g**, and **2j**) with halogens in *meta*- or *para*-positions were significantly less active than the diarylacetylenes with *ortho*-halogens. Once again, these

results are in consistent with the SAR findings in the stilbene family that also repress c-myc expression[2]. Finally, the N-methylation pattern in the diarylacetylenes suggested that N-methyl and N,N-dimethylaniline subunits led to equipotent repression of c-myc expression (*i.e.*, IC_{50} of **2b** ≈ IC_{50} of **2m**) but the desmethyl analog was considerably less active (IC_{50} of **2a** = 55±7.8 nm).

In summary, diarylacetylenes **2** have a dramatic effect on the proliferation of LS174T colon cancer cells by altering the expression of c-myc and thereby inducing p21(Wif1/Cip1). These results are consistent with similar findings using halogenated stilbenes[2] and suggest that diarylacetylenes and stilbenes repress colon cancer proliferation through similar mechanisms. Examining this question in detail is the subject of on-going investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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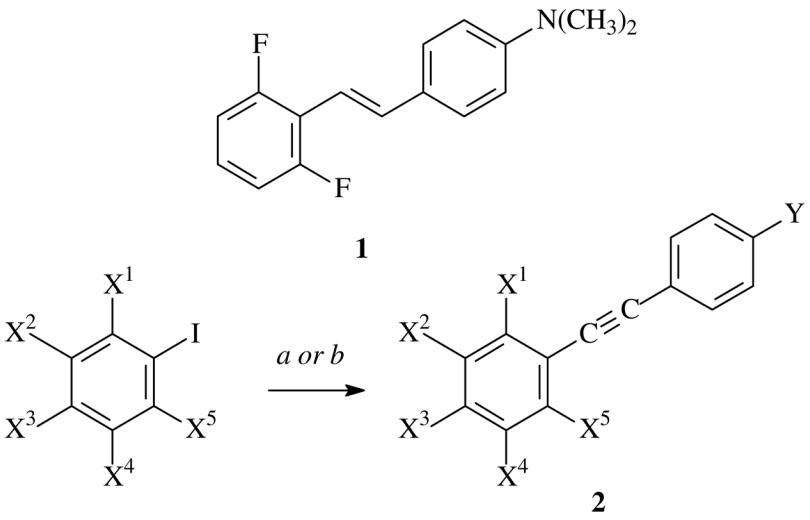

Figure 1. Synthesis of halogenated diarylacetylenes 2

Reagents: *a*, $HC{\equiv}CHC_6H_4Y$; 0.5% $Pd(PPh_3)_4$, 1% CuI, H_2O, 75°C, 1–2 h; *b*, *a*, $HC{\equiv}CHC_6H_4NH_2$; 0.5% $Pd(PPh_3)_4$, 1% CuI, H_2O, 75°C, 1–2 h followed by CH_3I, K_2CO_3, acetone, 5 h, 56°C.

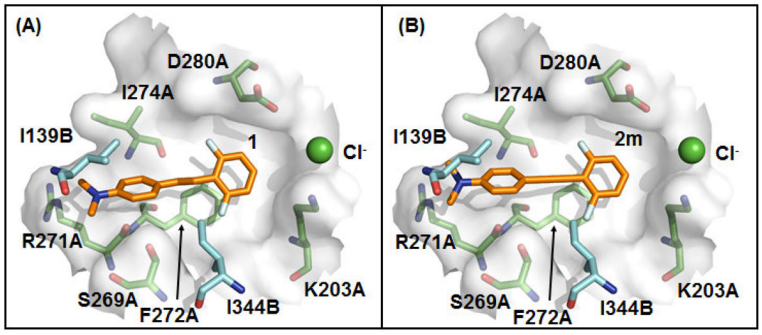

Figure 2. Docked structures of MAT2A binding with stilbene 1 (Panel A) and diarylacetylene 2m (Panel B)

The letters A and B following the amino acid designations refer to the individual oligomers in the MAT2A homodimer.

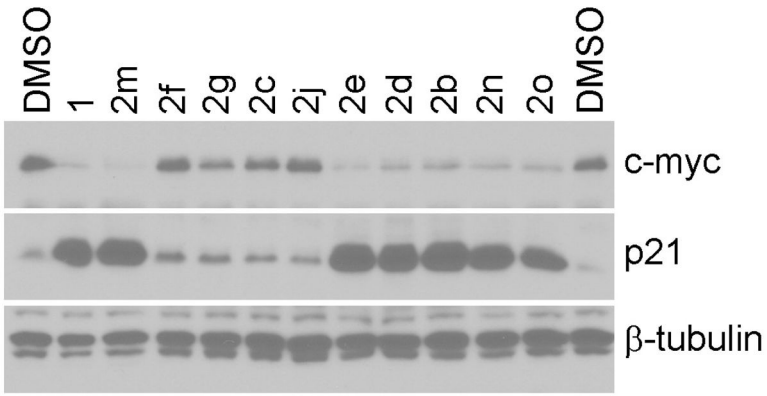

Figure 3. Repression of c-myc expression and induction of p21(Wif1/Cip1) by diarylacetylenes 2 in colon cancer cells

LS174T cells were treated with 1 μM of each diarylacetylenes **2** for 36 h. DMSO and **1** were used as control. Cell lysates were analyzed by western blotting with β-tubulin as a loading control.

Table 1

Halogenated N,N-diarylacetylenes 2 and their IC_{50} values in the inhibition of LS174T cell proliferation.

Compound	X^1	X^2	X^3	X^4	X^5	Y	Inhibition of LS174T Cell Proliferation IC_{50} (nM)
1	F				F	$N(CH_3)_2$	59±7.5
2a	F				F	NH_2	55±7.8
2b	F				F	$NHCH_3$	23±10.3
2c						$N(CH_3)_2$	>3000
2d	F					$N(CH_3)_2$	39±6.0
2e	Cl					$N(CH_3)_2$	31±3.1
2f		F				$N(CH_3)_2$	>3000
2g			F			$N(CH_3)_2$	>3000
2h	F	F				$N(CH_3)_2$	119±4.6
2i	F		F			$N(CH_3)_2$	56±8.1
2j		F	F			$N(CH_3)_2$	>3000
2k		F		F		$N(CH_3)_2$	>3000
2l	F			F		$N(CH_3)_2$	55±6.0
2m	F				F	$N(CH_3)_2$	23±6.0
2n	F				Cl	$N(CH_3)_2$	19±5.0
2o	Cl				Cl	$N(CH_3)_2$	52±7.1