

# Synthesis and Structure–Activity Relationship of Diarylamide Derivatives as Selective Inhibitors of the Proliferation of Human Coronary Artery Smooth Muscle Cells

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Received 13 October 2000; accepted 8 December 2000

**Abstract**—A series of diarylamide derivatives were synthesized and evaluated for their inhibitory activities against human coronary artery smooth muscle cells (SMCs) and human coronary artery endothelial cells (ECs). Compound **2w** was superior to the lead compound, Tranilast, in terms of the potency of the activity and cell selectivity. © 2001 Elsevier Science Ltd. All rights reserved.

## Introduction

Proliferation and migration of smooth muscle cells (SMCs) play a pivotal role in restenosis and in progression of atherosclerosis.<sup>1</sup> Arterial injury results in the migration of SMCs into the intimal layer of the arterial wall, where they proliferate and synthesize extracellular matrix components. It has been reported that many growth factors induce the proliferation of SMCs in vitro and in vivo.<sup>2</sup> Among them, platelet-derived growth factor (PDGF) is an important regulator of SMCs behavior through its well-defined actions as a potent chemoattractant and a strong mitogen.<sup>3</sup> Recently, Tranilast has been reported to markedly inhibit the proliferation and migration of SMCs and ECs,<sup>4</sup> and it showed a potent preventive effect for restenosis after PTCA in clinical study.<sup>5</sup> On the other hand, it has been reported that the selective inhibition of the proliferation of SMCs for ECs was preferable for the treatment of restenosis.<sup>6</sup> So we tried to search for more potent and SMCs selective compounds by the modification of Tranilast.

## Chemistry and Biology

The synthesis of diarylamide derivatives **2a–2ad**<sup>7</sup> were outlined in Scheme 1.

Acid-catalytic ester formation with sulfuric acid or amide formation with diphenylphosphoryl chloride (DPPCl) as a condensation reagent of 4,5-dimethoxy-2-nitro benzoic acid, followed by catalytic hydrogenation with 5% Pd/C gave the corresponding compounds **3c–f** in a good yield (70–80%). Other substituted anthranilic amides and esters were given in the same way. The substituted benzoyl chloride **6** were prepared from the corresponding benzoic acid **5** with thionyl chloride. Compounds **6** were used without purification. The condensation of **3** with **6** in the presence of triethylamine afforded **2a, 2b, 2e–ad** in a good yield (70–90%). In the case of hydroxy groups containing compounds, the hydroxy groups were protected as acetoxy groups before this condensation. Compounds **2c** and **2d** were prepared from the corresponding aniline **3a** or **3b** with 3,4-dimethoxy benzoyl chloride. Basic hydrolysis of **2d** provided **2e**.

A series of these compounds were evaluated for their inhibitory activities on PDGF-induced proliferation of

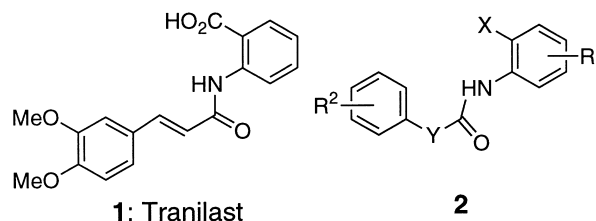
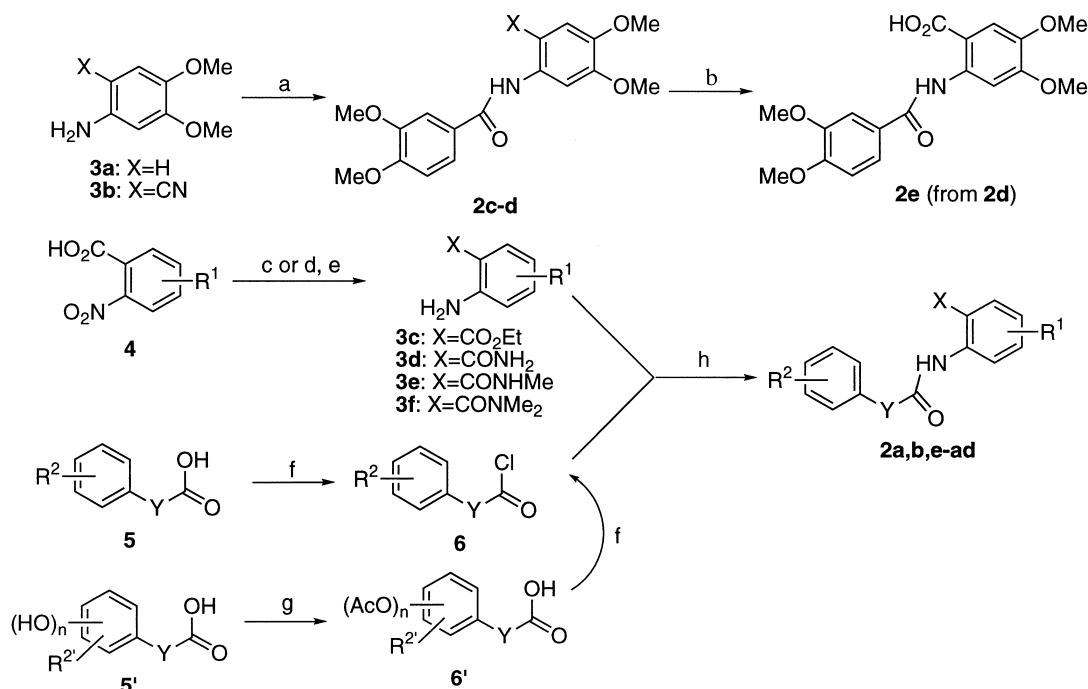


Figure 1. Structure of Tranilast and its derivatives.

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**Scheme 1.** Synthesis of compounds **2a–2ad**. Reagents and conditions: (a) 3,4-(OMe)<sub>2</sub>-PhCOCl or 3,4,5-(OMe)<sub>3</sub>-PhCOCl, Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub>; (b) NaOH/MeOH; (c) H<sub>2</sub>SO<sub>4</sub>/EtOH; (d) DPPCl, Et<sub>3</sub>N/CHCl<sub>3</sub> then NH<sub>3</sub> or MeNH<sub>2</sub> or Me<sub>2</sub>NH; (e) H<sub>2</sub>, 5% Pd/C/EtOH; (f) SOCl<sub>2</sub>/CHCl<sub>3</sub>, reflux; (g) Ac<sub>2</sub>O, Et<sub>3</sub>N/DMF; (h) Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub>.

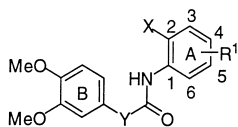
SMCs and FBS-induced proliferation of ECs. Inhibition of proliferation of these cells was determined by <sup>3</sup>H-thymidine incorporation as a previously reported method with a minor modification.<sup>8</sup>

### Results and Discussion

The inhibitory activities of **2a–ad** on PDGF-induced proliferation of SMCs are shown in Tables 1–3.

Compound **2k** (dimethoxy groups at R<sup>1</sup> and ethoxy carbonyl group at X moiety) showed more potent activity than Tranilast and its related compounds (**2a**, **2b**). The distance between the ring A and the ring B did not influence the activity (**2f**, **2j**, **2k**). In the case of dimethoxy substitution at R<sup>1</sup>, other groups at X moiety such as hydrogen, cyano, carboxyl and substituted carbonyl groups were less active (**2c**, **2d**, **2e**, **2h**, **2i**), but the carbamoyl group (**2g**) showed almost the same activity as the ethoxy carbonyl group (**2f**). For the

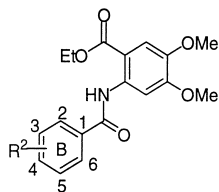
**Table 1.** IC<sub>50</sub> values of the compounds **2a–k** for inhibition of proliferation of SMCs and ECs

No.	R <sup>1</sup>	X	Y			
				SMCs IC <sub>50</sub> (μM) <sup>a</sup>	ECs IC <sub>50</sub> (μM) <sup>b</sup>	[ECs]/[SMCs]
Tranilast	H	CO <sub>2</sub> H	(E)-CH=CH	24.5	19.1	0.8
<b>2a</b>	H	CO <sub>2</sub> Et	(E)-CH=CH	>50	9.5	
<b>2b</b>	H	CONH <sub>2</sub>	(E)-CH=CH	16.7	34.5	2
<b>2c</b>	4,5-(OMe) <sub>2</sub>	H	—	>2.0	nt	
<b>2d</b>	4,5-(OMe) <sub>2</sub>	CN	—	>2.0	nt	
<b>2e</b>	4,5-(OMe) <sub>2</sub>	CO <sub>2</sub> H	—	>2.0	nt	
<b>2f</b>	4,5-(OMe) <sub>2</sub>	CO <sub>2</sub> Et	—	0.85	6	7
<b>2g</b>	4,5-(OMe) <sub>2</sub>	CONH <sub>2</sub>	—	0.72	>10	>14
<b>2h</b>	4,5-(OMe) <sub>2</sub>	CONHMe	—	>2.0	nt	
<b>2i</b>	4,5-(OMe) <sub>2</sub>	CONMe <sub>2</sub>	—	>2.0	nt	
<b>2j</b>	4,5-(OMe) <sub>2</sub>	CO <sub>2</sub> Et	CH <sub>2</sub>	0.8	4.7	6
<b>2k</b>	4,5-(OMe) <sub>2</sub>	CO <sub>2</sub> Et	(E)-CH=CH	1.2	4.1	4

nt: not tested.

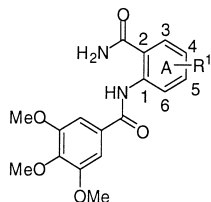
<sup>a</sup>Inhibitory activity against the proliferation of SMCs induced by PDGF-BB (20 ng/mL).

<sup>b</sup>Inhibitory activity against the proliferation of ECs induced by 5% FBS.

**Table 2.** IC<sub>50</sub> values of the compounds **2d**, **2l–x** for inhibition of proliferation of SMCs and ECs

No.	R <sup>2</sup>	SMCs IC <sub>50</sub> (μM) <sup>a</sup>	ECs IC <sub>50</sub> (M) <sup>b</sup>	[ECs]/[SMCs]
<b>2f</b>	3,4-(OMe) <sub>2</sub>	0.85	6	7
<b>2l</b>	4-OMe	0.61	<2.0	
<b>2m</b>	2-OMe	0.8	3.8	5
<b>2n</b>	3-OMe	1.6	6.1	4
<b>2o</b>	4-OAc	1.3	5.2	4
<b>2p</b>	3,4-(OAc) <sub>2</sub>	2.2	nt	
<b>2q</b>	3-OMe-4-OAc	2.8	nt	
<b>2r</b>	3-NO <sub>2</sub> -4-OH	1.8	7.2	4
<b>2s</b>	3-NH <sub>2</sub> -4-OH	>2.0	nt	
<b>2t</b>	3,5-(OMe) <sub>2</sub> -4-OAc	3.1	nt	
<b>2u</b>	3,4,5-(OAc) <sub>3</sub>	>2.0	nt	
<b>2w</b>	3,4,5-(OMe) <sub>3</sub>	0.31	4.6	15
<b>2x</b>	2,3,4-(OMe) <sub>3</sub>	0.41	2.9	7

nt: not tested.

<sup>a</sup>Inhibitory activity against the proliferation of SMCs induced by PDGF-BB (20 ng/mL).<sup>b</sup>Inhibitory activity against the proliferation of ECs induced by 5% FBS.**Table 3.** IC<sub>50</sub> values of the compounds **2y–z**, **2aa–ad** for inhibition of proliferation of SMCs and ECs

No.	R <sup>1</sup>	SMCs IC <sub>50</sub> (μM) <sup>a</sup>	ECs IC <sub>50</sub> (μM) <sup>b</sup>	[ECs]/[SMCs]
<b>2y</b>	H	>2.0	nt	
<b>2z</b>	4,5-F <sub>2</sub>	2	>10	>5
<b>2aa</b>	5-NO <sub>2</sub>	1.6	<2.0	
<b>2ab</b>	5-NH <sub>2</sub>	>2.0	nt	
<b>2ac</b>	5-Me	>2.0	nt	
<b>2ad</b>	4-Cl	>2.0	nt	

nt: not tested.

<sup>a</sup>Inhibitory activity against the proliferation of SMCs induced by PDGF-BB (20 ng/mL).<sup>b</sup>Inhibitory activity against the proliferation of ECs induced by 5% FBS.

number of methoxy group substitutions in ring B, the order of the potency was 3 (**2w**, **2x**) > 2 (**2f**) > 1 (**2l**, **2m**, **2n**), and the position of it was *para* (**2l**) > *ortho* (**2m**) > *meta* (**2n**). Polar groups like hydroxy groups (acetoxyl groups) and amino groups on ring B instead of methoxy groups resulted in decreased activity. Other substituents at R<sup>1</sup> such as difluoro, nitro, amino, methyl, and chloro groups are less active (Table 3). Among these compounds, **2w** was the most potent (IC<sub>50</sub> = 0.31 μM), and was about 80-fold more potent than Tranilast. The compounds with IC<sub>50</sub> values less than 2.0 μM were further evaluated against the proliferation of ECs. IC<sub>50</sub> values of **2w** for SMCs and for ECs were 0.31 and 4.6 μM, respectively, displaying selectivity about 15 times for SMCs. In contrast, IC<sub>50</sub> values of Tranilast were 24.5 and 19.1 μM, respectively, displaying selectivity about 0.8 times for SMCs. So, compound **2w** was superior to Tranilast in the strength of the activity and cell selectivity. The mechanism of the action of these derivatives has not been determined. Although several researchers have reported the mechanism of Tranilast,<sup>9</sup> the main mechanism remained unclear. So the definition of main target molecules for these compounds and the structural optimization are underway.

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