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Identification of a Novel Antiangiogenic Agent; 4-(*N*-Imidazol-2-ylmethyl)amino Benzopyran Analogues

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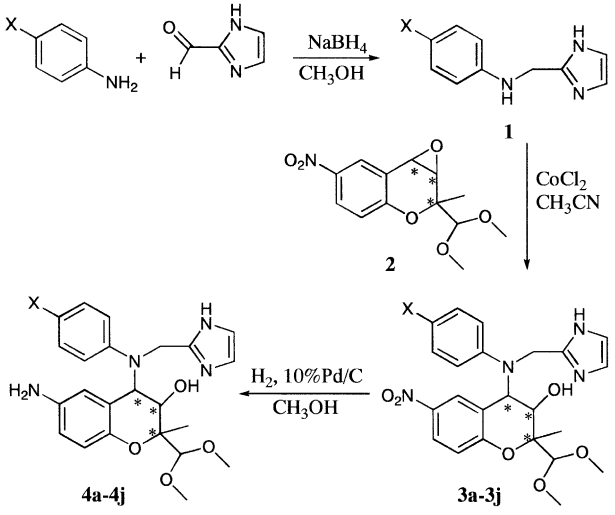
Abstract—A series of 4-(*N*-imidazol-2-ylmethyl)aminobenzopyran analogues, originally designed as K_{ATP} openers for ischemic diseases, showed antiangiogenic properties through the inhibition of HUVEC tube formation. Especially one of *p*-Cl substituted analogues (**4c**) completely inhibited HUVEC tube formation at 10 μM. The compound **4c** significantly inhibited tumor growth by 52% on A549 (human non small cell lung carcinoma) in nude mice xenografts without any significant side effects.

Angiogenesis plays critical roles in the pathophysiology of ischemic and neoplastic disorders, the most common causes of mortality, as wells as other chronic diseases including age-related macular degeneration, chronic lung diseases, neovascular retinopathies, and rheumatoid arthritis.[1] While we have been working on the identification of ATP sensitive potassium channel (K_{ATP}) openers[2] targeting ischemic diseases such as myocardial infarction and stroke, some of the compounds related to K_{ATP} showed the inhibitory effects on HUVEC (human umbilical vein endothelial cell) tube formation unexpectedly, indicating antiangiogenic properties. We prepared that series of compounds, and examined their inhibitory effects on HUVEC tube formation, followed by in vivo antitumor activity.

The imidazole analogue of 4-(*N*-aryl)-substituted benzopyran (BMS-191095) has been reported as a cardioselective K_{ATP} opener.[3] We prepared the same analogues modified at the 2-position of benzopyran from gem dimethyl to the acetal, and at the 6-position from nitrile to the nitro or amino group. *N*-(1*H*-imidazol-2-ylmethyl)anilines (**1**) were prepared by the reductive amination of 2-imidazolecarboxaldehyde with various 4-substituted anilines using $NaBH_4$, which were reacted with an optically pure benzopyran epoxide,[4] in the presence of $CoCl_2$ in CH_3CN to provide the 4-(*N*-imidazol-2-ylmethyl)amino-6-nitrobenzopyrans (**3a–3j**). 6-Aminobenzopyran derivatives (**4a–4j**) were obtained by the catalytic hydrogenation of the 6-nitro derivatives **3a–3j** (Scheme 1).



Scheme 1. Preparation of 4-(*N*-imidazol-2-ylmethyl)aminobenzopyrans.

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As shown in Table 1, most *p*-Cl substituted analogues (**3a–3d**, **4a–4c**) demonstrated inhibitory effects on HUVEC (primary cultured cells within passage 5 on Matrigel) tube formation at 50 μM concentration, respectively. The reduced 6-amino derivatives (**4a–4c**) seemed to be more potent than the 6-nitro compounds (**3a–3d**). Especially, the compound **4c** completely inhibited the tube formation at 50 μM, and even at 10 μM. At those concentrations, the compound **4c** didn't show any cytotoxicity on HUVECs. Other *p*-substituted analogues, both 6-nitro and 6-amino, demonstrated weaker activities than Cl-substituted compounds.

Although there are many therapeutic opportunities for the use of antiangiogenic inhibitors in clinic, targeting cancer has been most extensively investigated.[5] A growing tumor needs an extensive network of capillaries to provide nutrients and oxygen. In addition, the intratumoral blood vessels provide a way for tumor cells to enter the circulation and to metastasize to distant organs.[6] Therefore, angiogenesis is a crucial step in tumorigenesis. Approximately 75–80% of lung carcinomas are non-small cell lung carcinomas (NSCLC), of which prognosis remains poor, especially in advanced diseases.[7] It is suggested that it will be difficult to obtain better results in advanced NSCLC by chemotherapy alone, and novel treatment modalities are urgently needed including antiangiogenesis therapy. Then we investigated the effect of the compound **4c** on the growth of A549 (human NSCLC) in nude mice xenograft experiments, primarily. The compound **4c** was injected intraperitoneally at 50 mg/kg once a day from day 1 to day 20 after A549 implantation in BALB/c-nu/nu.

No mice were died in both control and treated group during experiments. As shown in Table 2, retardation or loss of weight gain was not observed by the treatment of **4c**. The compound **4c** significantly inhibited A549 NSCLC growth from 45 days to 61 days after implantation by 49–52%. The fundamental goal of antiangiogenic therapy is to return foci of proliferating microvessels to their resting state, and to prevent their re-growth.[7] The inhibition by **4c** was maintained until 61 days without any significant side effects, which gives the prospective that the compound **4c** may prevent the re-growth of microvessels and tumor.

Further we examined the anti-ischemic effects of the compounds to find out if those have K_{ATP} channel opening properties or not, according to the published procedures.[4,9]

As represented in Table 3, the compound **3b** showed good cardioprotective effects both in vitro and in vivo, while its vasorelaxant effect was comparably weak (IC_{50} >30 μM). But the compound **4c** neither showed any cardiopretectve effect nor vasorelaxation. Then, the antiangiogenic effects of this series of compounds may not be related with the K_{ATP} opening properties. The compound **4c** showed antiangiogenic properties without any K_{ATP} channel opening effects in this study.

Table 1. Inhibitory effects on HUVEC tube formation

X	Stereo (2, 3, 4)	Compd (50 μM)	Inhibition[a]	Compd (50 μM)	Inhibition
Cl	SRS	**3a**	+	**4a**	+ +
	SSR	**3b**	+ +	**4b**	+ +
	RSR	**3c**	+ +	**4c**	+ + +
	RRS	**3d**	+	**4d**	na
OCH_3	SRS	**3e**	+	**4e**	na
	RSR	**3f**	+	**4f**	+
CF_3	RSR	**3g**	na	**4g**	+
Br	RSR	**3h**	na	**4h**	+
OCF_3	SRS	**3i**	+	**4i**	na
	RSR	**3j**	na	**4j**	+

[a]–, control; +, inhibition; + +, significant inhibition; + + +, Tubes were not formed; na, not assayed.

Table 2. Antitumor efficacy of the compound **4c** on A549[a] human non-small cell lung carcinoma in nude mice xenografts

		1 day	14 day	25 day	35 day	45 day	61 day
Body weight[b] (g)	Control	22.50±0.34	25.16±0.26	25.43±0.45	25.88±0.48		
	4c treated[c]	22.30±0.29	25.42±0.30	26.17±0.38	27.10±0.42		
Tumor volume[d] (mm^3)	Control			152.9±28.7	308.6±54.8	483.9±105.0	1034.9±183.0
	4c treated			92.2±20.9	186.8±36.4	244.3*±47.0	494.8*±66.7
	Inh. (%)			39.7	39.5	49.5	52.2

[a]A549 was implanted sc into the right flanks of 8-week old nude mice (BALB/c-nu/nu).
[b]Each group consisted of 8 mice. Values represent mean±S.E.
[c]Nude mice were injected ip with **4c** (50 mg/kg) or vehicle (phosphate buffered saline containing 0.5% tween80), once daily from day 1 to day 20 after implantation.
[d]Significance was determined by student *t* test ($p<0.05$). Inhibition (%) was calculated as $(1-T/C)\times100$, where T and C were the mean tumor volume of treated and control group, respectively.

Table 3. Vasorelaxant potencies and cardioprotective effects

	Vaso[a] IC_{50} (μM)	In vitro cadioprotection (10 μM)[b]				In vivo anti-infarction[c] (0.3 mg/Kg) (IZ/AAR%/AAR/LV%)
		LVDP×HR (%)	EDP (mmHg)	TTC (min)	LDH (U/g)	
vehicle		23.0	43.4	20.3	29.9	61/40
3b	>30	55.7	14.0	28.0	10.7	41/33
4c	>30	19.3	62.3	23.5	na[d]	na[d]

[a]Vasorelaxant potency was assessed by measurement of IC_{50} for inhibition of methoxamine-contracted rat aorta. IC_{50} value is represented as a mean of 3 experiments.
[b]Cardioprotective effects were evaluated by measuring the contractile function (LVDP×HR), TTC, and LDH in the globally ischemic rat heart. Each value is an average of 3 experiments.
[c]In vivo anti-infarction effects were determined by measuring a ratio of myocardial infarct zone to area at risk (IZ/AAR) in ischemic myocardium damage rat model (0.3 mg/kg), $n=3$. Each value given is an average and within ±10%.
[d]Not assayed.

From this study we identified a novel chemical class[8] of angiogenesis inhibitors originally designed as a K_{ATP} opener targeting ischemic diseases, and confirmed its in vivo antitumor activity. We are going to prepare more analogues of the compound **4c** and investigate their antiangiogenic potencies to study the structure–activity relationships of this novel class of compounds, and to find more potent compounds. In addition, we will continuously study the antitumor activity of the compound **4c** through the optimization of dosing schedule, extension of tumor cell lines, and the combination with chemotherapeutic agents as well as its mechanism on antiangiogenic activity, pharmacokinetic profiles, and toxicity.

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