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6-ARYLAMINO-5,8-QUINOLINEDIONES AND 7-ARYLAMINO-5,8-ISOQUINOLINEDIONES AS INHIBITORS OF ENDOTHELIUM-DEPENDENT VASORELAXATION

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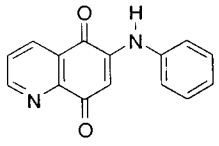
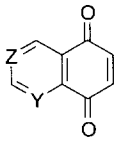
Abstract: 6-Arylamino-5,8-quinolinediones **3** and 7-arylamino-5,8-isoquinolinediones **4** were synthesized as inhibitors of endothelium-dependent vasorelaxation. The quinones inhibited the vasorelaxation of rat aorta with the endothelium. Among them, the quinones **3a**, **3b**, **3f**, **4b**, **4d** and **4g** strongly inhibited the vasorelaxation.

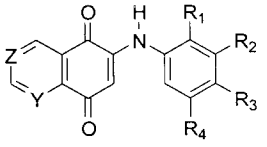
Quinones such as 6-phenylamino-5,8-quinolinedione[1-4] (LY83583, **1**), 1,4-naphthoquinones[5] and 9,10-phenanthraquinone[5] inhibit nitric oxide synthase (NOS). LY83583 (**1**) is an inhibitor of endothelial NO-dependent vasorelaxation and lowers intracellular cGMP in several tissues[1,3]. Muelsch *et al*[1] reported that **1** inhibits the release of EDRF (=NO), but no description about the inhibition of NOS by **1** was shown. Luo and Vincent[2] and Kumagai *et al*[4] have found that **1** inhibits NOS activity. **1** has been frequently used as an experimental tool to investigate the biological significance of NO. From this information, several bioisosteres of **1** including 6-arylamino-5,8-quinolinediones **3**, 7-arylamino-5,8-isoquinolinediones **4** and 2-arylamino-1,4-naphthoquinones **5** were synthesized and compared their biological activities with **1** (**Scheme 1**).



LY83583 (1)



2 : Y, Z=C or N



R_1, R_2, R_3, R_4= H, F, Cl, ..
3 : Y=N, Z=C
4 : Y=C, Z=N
5 : Y=Z=C

Scheme 1

We report the synthesis and inhibitory activities of quinones **2**, **3**, **4** and **5** on the endothelium-dependent vasorelaxation. A variety of quinones with different structures or substituents could inhibit the vasorelaxation

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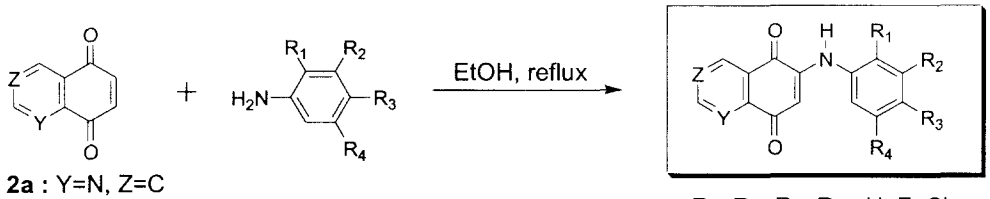
with different pattern. The quinones **3, 4** and **5** were incorporated with fluorine, chlorine or bromine respectively at the phenylamino group of the quinones to vary pharmacological properties. Indeed, the fluorine incorporation instead of hydrogen often improves pharmacological activities[6], since fluorine closely mimics hydrogen with respect to steric requirements at receptor sites and leads to enhancement of lipid solubility.

Synthesis: A method for the synthesis of the quinones **3, 4** and **5** is shown in **Scheme 2**. 6-Arylamino-5,8-quinolinediones **3a-3k (Table 1)** were synthesized by nucleophilic substitution of 5,8-quinolinedione (**2a**) with appropriate arylamines in the presence of Ce(III) according to the known method[7].



Scheme 2

7-Arylamino-5,8-isoquinolinediones **4a-4h (Table 1)** were synthesized by the substitution of 5,8-isoquinolinedione[8](**2b**) with corresponding arylamines. Experimental details for this procedure are cited in the **References and Notes**[9]. 1,4-Naphthoquinones **5a-5d** were also prepared as the similar method[7].

Biological Activities: The quinones were tested for their inhibitory activities on acetylcholine (ACh)-induced vasorelaxation of phenylephrine (PE)-precontracted rat aortas with the intact endothelium according to the procedure described in the reference 1 (**Table 1**). **1** and N^G-nitro-L-arginine (L-NA)[10], which are inhibitors of the endothelium-dependent vasorelaxation, were used as standard agents. EC_{50} value denotes the ACh concentration producing 50 percent of the vasorelaxation in the presence of quinones and E_{max} value represents the percent of the maximal vasorelaxation.

At the test concentration of 0.1 μM, the 5,8-quinolinediones **1, 2a** and most of **3a-3k** inhibited the ACh-induced vasorelaxation and increased the EC_{50} value for ACh, whereas L-NA repressed the vasorelaxation at the higher concentration (1 μM). Among them, **2a**, **3a**, **3b** and **3f** significantly reduced the maximal effect (E_{max}) of ACh. The four quinolinediones also greatly increased EC_{50} values by 5.2 ~ 9.4 times as the control values, while LY83583 (**1**) increased the EC_{50} value by 3.4 fold.

Most of the 5,8-isoquinolinediones **4a-4h** at the concentration of 0.1 μM inhibited the vasorelaxation and increased the EC_{50} value in the range of 0.190 to 1.130 μM. Among them, **4b**, **4d** and **4g** significantly reduced the E_{max} of ACh. The three isoquinolinediones also significantly reduced the ACh potency for the vasorelaxation to the similar level obtained by the higher concentration (1 μM) of L-NA, indicating that these quinones could act as more potent inhibitors than L-NA in the vasorelaxation potential.

Table 1. The inhibitory effects of the quinones on ACh-induced vasorelaxation of the rat aorta

Compound	Substituent				EC_{50} [a] (μM)	(N) [b]	E_{max} [a] (%)
	R_1	R_2	R_3	R_4			
Control					0.100 ± 0.018	(11)	100 ± 1
L-NA					1.100 ± 0.227*	(3)	69 ± 3*
1	H	H	H	H	0.335 ± 0.032*	(7)	77 ± 5*
2a					0.519 ± 0.208*	(3)	80 ± 3*
2b					0.374 ± 0.036*	(3)	92 ± 5
2c					0.127 ± 0.012	(3)	98 ± 5
3a	H	H	F	H	0.541 ± 0.179*	(4)	79 ± 4*
3b	H	F	H	H	0.805 ± 0.241*	(3)	85 ± 5*
3c	H	F	F	H	0.251 ± 0.047	(3)	92 ± 5
3d	H	F	H	F	0.449 ± 0.063*	(3)	91 ± 5*
3e	F	H	F	H	0.288 ± 0.016	(4)	94 ± 4
3f	F	F	F	H	0.937 ± 0.232*	(4)	72 ± 4*
3g	H	H	Cl	H	0.178 ± 0.025	(4)	86 ± 7*
3h	H	H	Br	H	0.302 ± 0.034*	(3)	88 ± 2*
3i	H	H	I	H	0.214 ± 0.019	(3)	89 ± 3*
3j	H	H	CF_3	H	0.607 ± 0.097*	(3)	91 ± 7*
3k	H	H	OCF_3	H	0.603 ± 0.051*	(3)	88 ± 4*
4a	H	H	H	H	0.439 ± 0.038*	(3)	93 ± 1
4b	H	H	F	H	1.230 ± 0.546*	(4)	72 ± 8*
4c	H	F	H	H	0.562 ± 0.048*	(3)	89 ± 4*
4d	H	F	F	H	0.640 ± 0.034*	(3)	81 ± 4*
4e	H	F	H	F	0.290 ± 0.070	(3)	94 ± 3
4f	F	H	F	H	0.430 ± 0.053*	(3)	96 ± 5
4g	F	F	F	H	1.130 ± 0.273*	(4)	87 ± 1*
4h	H	H	Cl	H	0.190 ± 0.032	(3)	96 ± 4
5a	H	H	H	H	0.239 ± 0.052	(3)	98 ± 1
5b	H	H	F	H	0.362 ± 0.029*	(3)	98 ± 4
5c	F	F	F	H	0.330 ± 0.041*	(3)	97 ± 1
5d	H	H	Cl	H	0.220 ± 0.017	(3)	98 ± 4

a) EC_{50} is the ACh concentration producing 50 percent of the vasorelaxation of PE-precontraction (0.3 μM) after preincubation of each quinone (0.1 μM) or L-NA (1 μM) for 20 min and E_{max} is the percent of the maximal ACh-induced vasorelaxation.

b) Data are means ± S.E.M. using N numbers of aortic rings from separate animals.

* Significantly different from the control group by student's t-test ($P < 0.05$).

In contrast, the 1,4-naphthoquinones **5a-5d** at the test concentration of 0.1 μM exhibited no or poor, if any, inhibitory activities on the ACh-induced vasorelaxation.

The quinones **2a**, **3**, **4** and **5** did not exert any inhibitory effect on the vasorelaxation in the aortic rings without the intact endothelium. This observation can be possibly explained by that the quinones interfere with the relaxant action of endogenously released NO, as indicated in the similar action[3] of LY83583 (**1**) on the phenylephrine-induced contraction.

In terms of structure-activity relationship, observations presented in **Table 1**, the quinolinedione skeletons **3a-3k** and isoquinolinedione skeletons **4a-4h** showed, in general, more potent inhibitory activities than the 1,4-naphthoquinone skeletons **5a-5d**. The 5,8-quinolinediones **3a**, **3b** and **3f**, containing a 6-(fluorinated-phenyl)amino group, inhibited strongly the vasorelaxation. Comparingly, **3c** and **3e** with the group showed moderate inhibitory activities. Among the 5,8-isoquinolinediones **4a-4h**, the 7-[(fluorinated-phenyl)amino]-5,8-isoquinolinediones **4b**, **4d** and **4g** also showed somewhat potent inhibitory activities. In addition, the quinones **2b**, **2b** and **2c** without an arylamino group exhibited the inhibitory activities. Thus, the arylamino moiety is not essential for the inhibitory activities of these quinones, but it partially improves the activities.

Conclusion: 5,8-Quinolinedione (**2a**), 6-arylamino-5,8-quinolinediones (**3b**, **3f**) and 7-arylamino-5,8-isoquinolinediones (**4b**, **4d** and **4g**) strongly inhibited the ACh-induced vasorelaxation of PE-precontracted rat aorta with the intact endothelium. Further pharmacological investigations of these quinones as inhibitors of endothelial and neuronal NO synthases are in progress.

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References and Notes

1. Muelsch, A.; Busse, R.; Lieb, S.; Fostermann, U. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 283.
2. Luo, D.; Vincent, S. R. *Europ. J. Pharmacol.* (Mol. Pharmacol. section), **1995**, *290*, 247.
3. Malta, E.; Macdonald, P. S.; Dusting, G. J. *Naunyn-Schmiedeber's Arch. Pharmacol.* **1988**, *337*, 459.
4. Kumagai, Y.; Midorikawa, K.; Nakai, Y; Yoshikawa, T.; Kushida, K.; Homma-Takeda, S.; Shimojo, N. *Europ. J. Pharmacol.* **1998**, *360*, 213.
5. Kumagai, Y.; Nakajima, H, Midorikawa, K.; Homma-Takeda, S.; Shimojo, N. *Chem. Res. Toxicol.* **1998**, *11*, 608.
6. Filler, R. In *Organofluorine compounds in medicinal chemistry and biomedical applications*; Filler, R. *et al*, Ed.; Elsevier Science; Paris, 1990; pp 1-22.
7. Pratt, Y. T. *J. Org. Chem.* **1962**, *27*, 3905.
8. Joseph, P. K.; Joullie, M. M. *J. Med. Chem.* **1964**, *7*, 801.
9. *General procedure for synthesis of 7-arylamino-5,8-isoquinolinediones* **4**: A solution of 5,8-isoquinolinediones **2b**[8] (0.01 mol) in 100 mL of 95% EtOH was added to a solution of an arylamine (0.011 mol) in 50 mL of 95% EtOH with stirring at RT for 2 hr and then refluxed for 5 hr. After the mixture was kept overnight, the precipitate was collected by the filtration. Crystallization from aq. EtOH afforded the 5,8-isoquinolinediones **4a-4h** (**Scheme 2** and **Table 1**).
10. Moncada, S.; Higgs, A.; Furchgott, R. *Pharmacol. Rev.* **1997**, *49*,137.