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2(1*H*)-Quinolinone derivatives as novel anti-arteriostenotic agents showing anti-thrombotic and anti-hyperplastic activities

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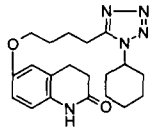
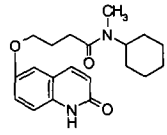
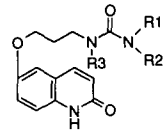
Abstract: In order to search for anti-arteriostenotic agents, a series of 2(1*H*)-quinolinone derivatives was synthesized and evaluated for anti-thrombotic activity and for anti-hyperplastic activity. From this series, (–)-6-[3-[3-cyclopropyl-3-[(1*R*,2*R*)-2-hydroxycyclohexyl]ureido]propoxy]-2(1*H*)-quinolinone (**1p**, OPC-33509) was selected as the best candidate by balancing the efficacy on anti-thrombosis and anti-hyperplasia.

Introduction: Ischemic diseases such as myocardial infarction,[1)] unstable angina[2)] and cerebral infarction[3)] are caused by an arteriostenosis which is led by chronically formed vascular intimal thickening and acutely formed thrombus in the vessels. Therefore, we decided to develop an anti-arteriostenotic agent showing both anti-thrombotic activity and anti-hyperplastic activity for the treatment of ischemic diseases.

We have developed cilostazol (launched in Japan, and submitted to FDA), a 2(1*H*)-quinolinone derivative with a tetrazole ring in the side chain, as an anti-thrombotic agent.[4)] However the anti-hyperplastic activity of this agent was weak. During the course of previous exploratory studies on anti-thrombotic agents, we also found cilostamide,[5)] which has an amide moiety in the side chain, as a potent platelet aggregation inhibitor. Moreover, this compound also has a potent anti-hyperplastic activity. Unfortunately, this compound was not further developed because of the side effect of tachycardia. We were therefore interested in the amide moiety of cilostamide, and tried to search for new potent compounds by the modification of cilostamide. In this paper, we describe the synthesis and activities of novel 2(1*H*)-quinolinone derivatives **1** with an incorporated urea moiety.


cilostazol


cilostamide



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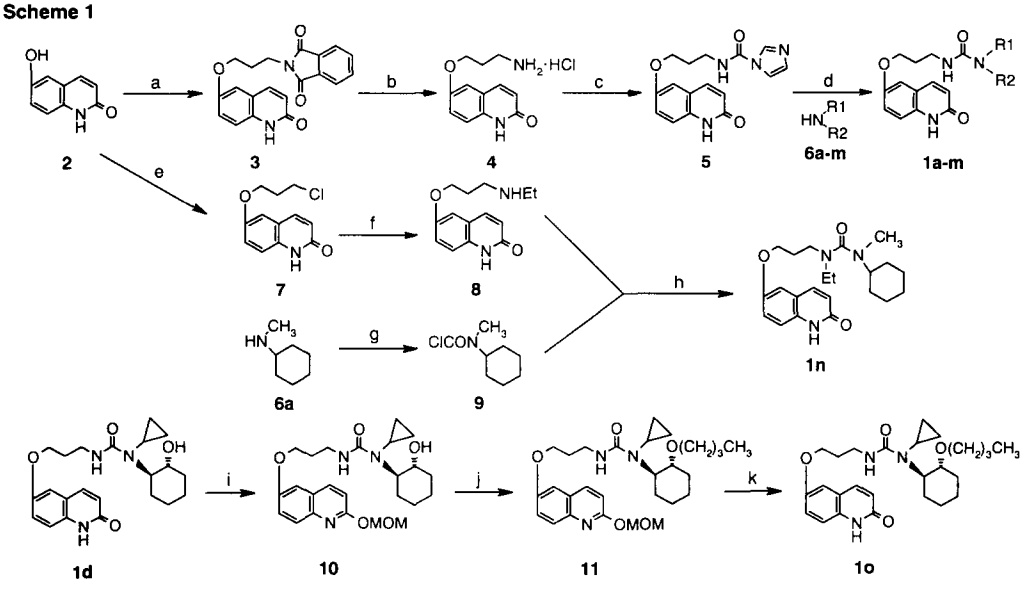
Synthesis: The target urea derivatives **1a-o** were prepared as outlined in **Scheme 1**. Alkylation of 6-hydroxy-2(1*H*)-quionolinone **2**[6] with *N*-(3-bromopropyl)phthalimide furnished an alkyl phthalimide **3** in good yield, and deprotection with $NH_2NH_2 \cdot H_2O$ provided the amine **4**. The imidazolyl urea **5** was obtained as a stable crystalline solid from the amine **4** by treating with 1,1'-carbonyldiimidazole and 2eq. imidazole in DMSO.[7] The trisubstituted urea derivatives **1a-m** were efficiently synthesized by coupling reactions of the imidazolyl urea intermediate **5** with various amines **6a-m**.

The tetrasubstituted urea derivative **1n** was prepared as follows. Alkylation of **2** with 1-bromo-3-chloropropane gave **7**, which was converted to an ethylamino derivative **8**. Chlorocarbonylation of *N*-methylcyclohexylamine **6a** using triphosgene gave **9** in quantitative yield. Condensation of **8** with **9** in the presence of potassium carbonate in DMF gave the tetrasubstituted urea **1n**.

The alkoxy cyclohexane derivative **1o** was synthesized from **1d** in three steps. Selective protection of the lactam in compound **1d** as a methoxymethyl ether gave a quinoline derivative **10**. Alkylation of **10** with n-butyl iodide gave **11**, and the resulting product was deprotected to give the alkyl ether **1o**.

Scheme 1



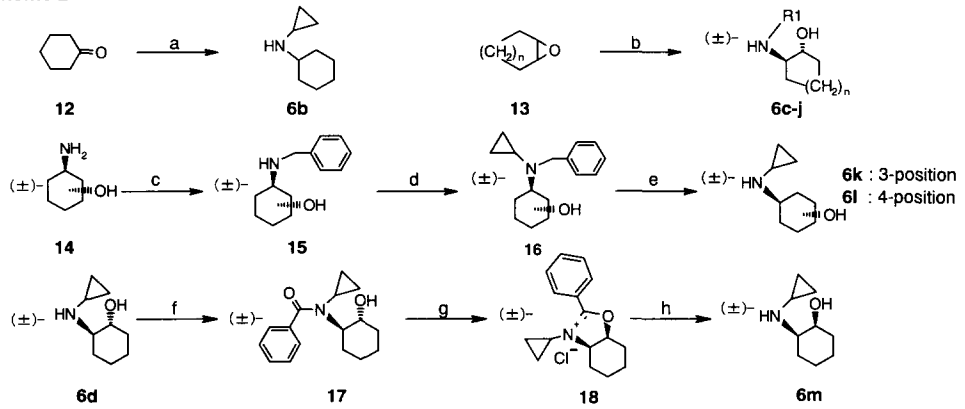
(a)*N*-(3-bromopropyl)phthalimide, 2eq. K_2CO_3 / DMF, 50°C (83%); (b)$NH_2NH_2 \cdot H_2O$ / EtOH, reflux (72%); (c)1,1'-carbonyldiimidazole, 2eq. imidazole / DMSO, r.t. (87%); (d)**6a-m**, reflux in $CHCl_3$ or 100°C in DMF (yields are shown in **Table 1**)

(e)1-bromo-3-chloropropane, K_2CO_3 / DMF (69%); (f)ethylamine / MeOH (58%); (g)triphosgene, pyridine / toluene (100%); (h)K_2CO_3 / DMF (57%)

(i)CH_3OCH_2Cl, $(iPr)_2NEt$ / CH_2Cl_2 (56%); (j)NaH, n-BuI / DMF (30%); (k)2N-HCl / MeOH (92%)

The preparation of the various amines **6b-m** is shown in **Scheme 2.**

Scheme 2



(a)cyclopropylamine / EtOH and then H_2, 10%Pd-C (61%); (b)n = 1, 2 :cycolpropylamine / EtOH, reflux (**6c** : 26%, **6d** : 81%); n = 3 :cycolpropylamine, $AlMe_3$ / CH_2Cl_2, r.t. (**6e** : 93%)[8]; n = 2 :methylamine / MeOH (**6f** : 89%); n = 2 :cyclopentylamine / EtOH (**6g** : 54%); n = 2 :cyclohexylamine / EtOH (**6h** : 45%); n = 2 :cycloheptylamine / EtOH (**6i** : 64%); n = 2 :cyclooctylamine / EtOH (**6j** : 43%);

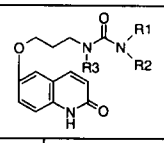
(c)benzaldehyde, $NaBH_4$ / EtOH (3-position : 57%, 4-position[9] : 50%); (d)[(1-ethoxycyclopropyl)oxy]trimethylsilane, $NaCNBH_4$, AcOH / MeOH[10] (3-position : 57%, 4-position : 50%); (e)H_2, 10%Pd-C / EtOH (**6k** : 57%, **6l** : 35%)

(f)benzoyl chloride, DMAP/ Pyridine (46%); (g)thionyl chloride; (h)10%aq.KOH / EtOH (72% 2steps).[11]

Results and Discussion: The pharmacological profiles of the synthesized 2(1*H*)-quinolinone derivatives **1a-o** are summarized in **Table 1.** At first, the inhibitory activities of the compounds were measured *in vitro* against adenosine diphosphate (ADP)- and collagen-induced blood platelet aggregation using rabbit platelet-rich plasma.[12] All compounds showed higher inhibitory activities than cilostazol, and aspirin was found to be inactive. The trisubstituted urea **1a** was about six times more active than tetrasubstituted urea **1n.** The order of the potency according to ring size of R_1 in the 2-hydroxycyclohexyl substituted analogues was found to be cyclooctyl (**1j**) > cycloheptyl (**1i**) > cyclohexyl (**1h**) > cyclopentyl (**1g**) > cyclopropyl (**1d**) > methyl (**1f**). Next, we assessed *in vivo* their anti-thrombotic activities on a pulmonary thromboembolism model in mice.[13] The compounds **1b** (R_1 = cyclopropyl, R_2 = cyclohexyl), **1d** (R_1 = cyclopropyl, R_2 = 2-*trans*-hydroxycyclohexyl), **1j** (R_1 = cyclooctyl, R_2 = 2-*trans*-hydroxycyclohexyl) and **1m** (R_1 = cyclopropyl, R_2 = 2-*cis*-hydroxycyclohexyl) showed higher inhibitory activities than cilostazol or cilostamide. The anti-thrombotic activities *in vivo* did not correlate to the aggregation inhibitory activities *in vitro*. We presumed that the discrepancy of the activities between *in vitro* and *in vivo* was caused by the difference of species and/or pharmacokinetics. Finally, to evaluate anti-hyperplastic activities *in vivo*, we developed a balloon injury model which evaluates the intimal hyperplasia by measurement of arterial DNA synthesis.[14] Using this model, **1d** (R_1 = cyclopropyl, R_2 = 2-*trans*-hydroxycyclohexyl) showed the most potent inhibitory activity on arterial DNA synthesis, whereas its *cis*-isomer **1m** showed low activity and the positional

isomers **1k** and **1l** had little or no activity. Based on their actions *in vivo*, 2-*trans*-aminoalcohol **1d** was selected as the best compound.

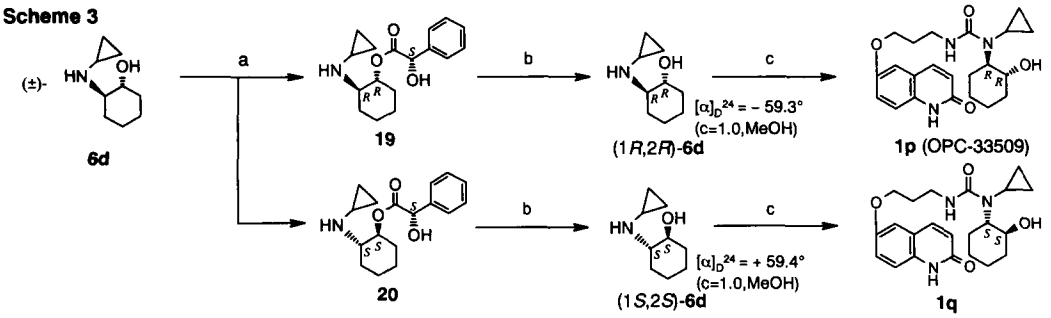
Table 1

compound				Yield (%)	*in vitro*		*in vivo*	
					inhibitory activity[a] IC_{50} (μM)		inhibition (%) at 30mg/kg, *p.o.*	
	R_1	R_2	R_3		ADP	collagen	*anti-thrombotic activity*[b]	*anti-hyperplastic activity*[c]
1a	CH_3		H	72	2.6	2.7	21	15
1b			H	64	2.2	2.4	100	15
1c			H	40	2.5	3.5	67	23
1d			H	72	2.5	3.1	100	29
1e			H	35	2.6	2.8	44	0
1f	CH_3		H	73	3.9	15	18	10
1g			H	50	0.27	1.8	78	20
1h			H	51	0.19	0.15	57	18
1i			H	53	0.12	0.030	69	11
1j			H	72	0.080	0.030	100	27
1k			H	56	3.0	2.8	20	0
1l			H	17	3.8	3.1	0	7
1m			H	58	2.3	3.3	100	19
1n	CH_3		Et	-	15	16	58	12
1o			H	-	0.71	3.5	33	19
cilostazol					29	31	82	12
cilostamide					2.4	2.2	84	42
aspirin					>1000	>1000	33	16

[a]Inhibitory activities against ADP- or collagen-induced rabbit platelet aggregation.
[b]Inhibitory activities on pulmonary thromboembolism model in mice.
[c]Inhibitory activities on balloon injury model in rats.

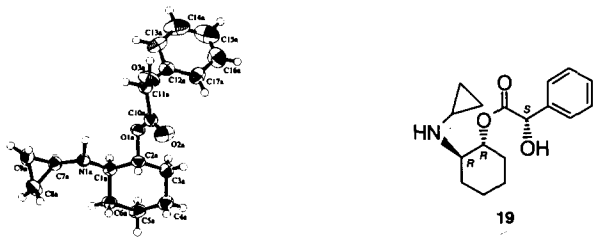
Since the 2-*trans*-aminoalcohol **1d** is a racemate, we prepared two optically active enantiomers in order to compare their activities. The resolution of a racemic amine **6d** was accomplished by separation of the (*S*)-mandelic acid esters **19**[15] and **20** on a silica-gel column, followed by hydrolysis of each ester. Condensation of each optically pure amine with the imidazolyl urea **5** gave **1p**[16] (OPC-33509) or **1q** (**Scheme 3**). The configuration of **19** was determined by a single-crystal X-ray analysis (**Figure 1**). Therefore, absolute stereochemistry of the corresponding **1p** was determined as (1*R*,2*R*).

Scheme 3



(a)(*S*)-mandelic acid, *p*-TsOH·H_2O / toluene and then column chromatography (**19** : 25%, **20** : 17%)
(b)10%aq.KOH / EtOH (100%); (c) **5** / $CHCl_3$ (**1p** : 63%, **1q** : 53%)

Figure 1 Perspective view and absolute stereochemistry of **19**



Pharmacological profiles of the enantiomers are summarized in **Table 2.**

Table 2

compound	*in vitro*		*in vivo*			
	inhibitory activity IC_{50} (μM)		inhibition (%) *p.o.*			
	ADP	collagen	*anti-thrombotic activity*		*anti-hyperplastic activity*	
			30mg/kg	10mg/kg	30mg/kg	10mg/kg
1p	2.3	2.2	100	88	24	26
1q	2.4	2.2	100	29	13	1

Both compounds showed equal platelet aggregation inhibitory activities *in vitro*. However, the (*R*,*R*)-isomer **1p** was more potent *in vivo* than the (*S*,*S*)-isomer **1q** on both anti-thrombotic activity and anti-hyperplastic activity at 10mg/kg p.o. dosing, and the two

activities were well balanced. The compound **1p** showed little systemic tachycardia and blood pressure change in dogs.

In summary, we have reported the synthesis and activities of novel 2(1*H*)-quinolinone derivatives, which possess an urea moiety, as anti-arteriostenotic agents. Among them, the compound **1p (OPC-33509)** has been selected as a candidate for further pharmacological and toxicological evaluations, and is expected to be a clinically useful anti-arteriostenotic agent for the treatment of ischemic diseases.

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14. The experimental methods of a balloon injury model in rats were modified from the following: Lamb, M. A.; Manning, J. E.; Reddick, R. L.; Griggs, T. R. *Arteriosclerosis* **1984**, *4*, 84. : One hour after oral administration of each compound, the left common carotid arteries of male SD rats (n = 7~8 / group) were injured using an embolelectomy 2-French balloon catheter. Next day, the compounds were administrated twice and on third day, one hour after administration of the compounds, the solution of [^{3}H]-thymidine (0.25 μCi/ml/kg) was injected intravenously to each rat. The rats were sacrificed 45 minutes later and 1cm length of left carotid arteries were cutoff, dissolved in 0.5 N NaOH, and neutralized with 5 N HCl. Radioactivity of the carotid were measured by using a liquid scintillation counter (Alaka Model LSC-900).

$$\text{Inh (\%)} = \frac{\text{L(c)} - \text{L(d)}}{\text{L(c)} - \text{R(c)}} \times 100$$

L(c): dpm(^{3}H-thymidine) / cm of left carotid artery in control group
R(c): dpm(^{3}H-thymidine) / cm of right carotid artery in control group
L(d): dpm(^{3}H-thymidine) / cm of left carotid artery in drug-administered group

15. Chemical data of **19** are as follows: Colorless columns recryst. from AcOEt-n-hexane; m.p. 101.0 - 103.0°C; $[\alpha]_D^{22}$ = +11.5° (c = 1.0, $CHCl_3$); ^{1}H-NMR (250 MHz, $CDCl_3$, δ ppm): 0.22 - 0.56 (4H, m), 1.02 - 1.36 (4H, m), 1.48 - 1.88 (4H, m), 2.00 - 2.24 (2H, m), 2.68 (1H, m), 3.56 (1H, br. s), 4.69 (1H, m), 5.16 (1H, s), 7.23 - 7.50 (5H, m).; IR (KBr, cm^{-1}): 3090, 2939, 2867, 1747, 1453, 1183, 1022, 991, 759, 705.
16. Chemical data of **1p** are as follows: White powder recryst. from EtOH; m.p. 160.5 - 162.0°C; $[\alpha]_D^{24}$ = –2.2° (c = 1.0, MeOH); ^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 0.52 - 0.84 (4H, m), 0.98 - 1.25 (3H, m), 1.45 - 1.76 (4H, m), 1.80 - 2.00 (3H, m), 2.43 (1H, m), 3.13 - 3.40 (3H, m), 3.74 (1H, m), 4.02 (2H, t, J = 6.0 Hz), 4.49 (1H, d, J = 5.0 Hz), 6.26 (1H, t, J = 5.0 Hz), 6.48 (1H, d, J = 9.5 Hz), 7.08 - 7.30 (3H, m), 7.83 (1H, d, J = 9.5 Hz), 11.62 (1H, br. s); IR (KBr, cm^{-1}): 3350, 2925, 1660, 1630, 1610, 1525, 1280, 1125, 855.