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Synthesis of 4-Alkoxy-2-phenylquinoline Derivatives as Potent Antiplatelet Agents

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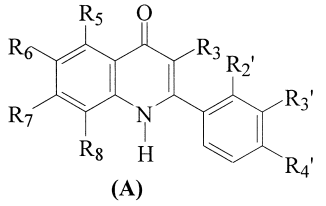
Abstract—In our continuing search for novel antiplatelet agents, 4-alkoxy derivatives of 2-phenylquinoline as well as related compounds were prepared. Through biological screening, a preliminary structure–antiplatelet activity relationship was established. Compounds 5-ethyl-4-methoxy-2-phenylquinoline (**8**), 4-ethoxy-5-ethyl-2-phenylquinoline (**9**), 4-ethoxycarbonylmethoxy-5-ethyl-2-phenylquinoline (**10**), 4-ethoxycarbonylbutoxy-5-ethyl-2-phenylquinoline (**12**) and 5-ethyl-4-(*N*-ethylcarboxido)methoxy-2-phenylquinoline (**17**) all demonstrated potent antiplatelet activity. Among them, compound **8** was the most potent with an IC_{50} value of 0.08 μM and was about 3-fold more active than indomethacin. The mechanism of antiplatelet action of **8** is possibly through its inhibition on cyclooxygenase or thromboxane synthetase. © 2001 Published by Elsevier Science Ltd.

Introduction

In a previous paper,[1–3] a series of mono- and disubstituted 2-phenyl-4-quinolone derivatives (A) were synthesized and their antiplatelet activities were evaluated.



(A)

R_3, R_5, R_6, R_7, R_8, $R_{2'}$, $R_{3'}$, $R_{4'}$ = H, Cl, F, OCH_3, alkyl

We were encouraged that all of the 5-alkyl derivatives of 2-phenyl-4-quinolone (**1**)[4] exhibited potent antiplatelet activity. In particular, 5-ethyl-2-phenyl-4-quinolone (**2**) had drawn our attention after showing an IC_{50} value of 0.15 μM that is more potent than indomethacin. In this work, we explored further into the analogues of compounds **1** and **2**, and prepared their *N*-alkyl and 4-alkoxy derivatives by alkylation. During the screening of their antiplatelet activity, we learned that some of the 4-alkoxy derivatives had excellent activity and summarized our finding in this report.

Results and Discussion

Chemistry

Target compounds, 4-alkoxy-2-phenylquinolines (**4**–**13**) and *N*-methyl-2-phenylquinolones (**14**, **15**), were synthesized according to Scheme 1. The preparation of two typical compounds, 5-ethyl-4-methoxy-2-phenylquinoline (**8**) and 5-ethyl-*N*-methyl-2-phenylquinolone (**15**), shown in Scheme 1, illustrated the general procedures.

First, 5-ethyl-2-phenylquinolone (**2**)[2] was methylated with methyl iodide and NaH in THF to form **8** (mp 81–82 °C) and **15** (mp 125–127 °C) in a ratio of 1:5.4. Elemental analysis and mass spectral data (*m*/*z* 263, M^+) established their molecular formulae as $C_{18}H_{17}NO$ for both compounds, suggesting that they were possibly the *O*-methyl and *N*-methyl isomers.

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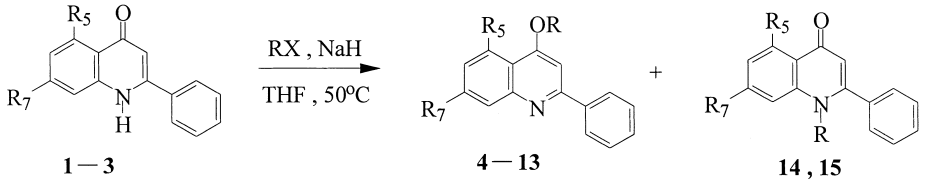


1 — 3 4 — 13 14 , 15

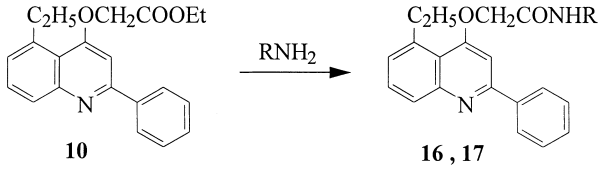
1 $R_5 = R_7 = H$
2 $R_5 = C_2H_5$, $R_7 = H$
3 $R_7 = C_2H_5$, $R_5 = H$
4 $R_5 = R_7 = H$, $R = CH_3$
5 $R_5 = R_7 = H$, $R = C_2H_5$
6 $R_5 = R_7 = H$, $R = CH_2COOEt$
7 $R_5 = R_7 = H$, $R = CH_2COOH$
8 $R_5 = C_2H_5$, $R_7 = H$, $R = CH_3$
9 $R_5 = C_2H_5$, $R_7 = H$, $R = C_2H_5$
10 $R_5 = C_2H_5$, $R_7 = H$, $R = CH_2COOEt$
11 $R_5 = C_2H_5$, $R_7 = H$, $R = (CH_2)_3COOEt$
12 $R_5 = C_2H_5$, $R_7 = H$, $R = (CH_2)_4COOEt$
13 $R_7 = C_2H_5$, $R_5 = H$, $R = C_2H_5$
14 $R_5 = R_7 = H$, $R = CH_3$
15 $R_5 = C_2H_5$, $R_7 = H$, $R = CH_3$

Scheme 1.

The ^1H NMR spectrum of **15** indicated the presence of a *N*-methyl signal at δ 3.56, a one-proton signal at 6.23 (H-3), the 5-ethyl signals at δ 1.15 and 3.50, and eight aromatic protons at δ 7.19 to 7.62 (m). The data led to the assignment of **15** as 5-ethyl-*N*-methyl-2-phenyl-4-quinolone. Similarly, the assignment of **8** as 5-ethyl-4-methoxy-2-phenylquinoline was based on its ^1H NMR spectral data, which included an *O*-methyl signal at δ 4.12, the 5-ethyl signals and eight aromatic proton signals.

However, when **2** was treated with ethyl iodide, only the corresponding *O*-ethylated product (**9**) was obtained. Likewise, treating **2** with various ethoxycarbonylalkyl halides afforded only the corresponding *O*-alkylated products (**10**–**12**). On the other hand, the alkylation of compound **1** and 7-ethyl-2-phenyl-4-quinolone (**3**) primarily gave only *O*-alkylated products, except for the methylation which again yielded both *N*- and *O*-methylated products.

When 4-ethoxycarbonylmethoxy-2-phenylquinoline (**6**) was treated with 10% NaOH, the hydrolyzed product 4-hydroxycarbonylmethoxy-2-phenylquinoline (**7**) was obtained. Finally, we also treated 4-ethoxycarbonylmethoxy-5-ethyl-2-phenylquinoline (**10**) with methylamine and ethylamine to provide their corresponding amide derivatives (**16**, **17**) (Scheme 2).



10 **16 , 17**

16 $R = CH_3$
17 $R = CH_2CH_3$

Scheme 2.

Antiplatelet activity

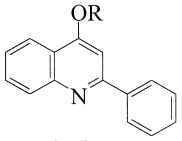
To obtain a broader picture of how our target compounds performed as antiplatelet agents, two selected compounds (**4** and **5**) were evaluated as inhibitors of platelet aggregation induced by thrombin, arachidonic acid (AA), collagen and PAF.[5,6] As seen from the results in Table 1, both **4** and **5** displayed very similar patterns of preferential inhibition. They were relatively strong inhibitors of AA- and collagen-induced platelet aggregation, but were poor inhibitors for thrombin- and PAF-induced aggregation. Accordingly, we narrowed down our focus to a comparison of AA-induced platelet aggregation inhibitory activity in the forthcoming discussion.

From the data in Table 2, the 4-alkoxy-2-phenylquinolines **4** and **5** with methoxy and ethoxy substitution, respectively, at C-4, displayed IC_{50} values of 2.1 and 2.0 μM and were 10 times more potent than aspirin. Subsequent replacement of the 4-alkoxy R group with $-CH_2COOCH_2CH_3$ (**6**) resulted in greatly reduced activity. Hydrolysis of the $-CH_2COOCH_2CH_3$ moiety to $-CH_2COOH$ yielded **7** and further reduced potency.

Next, we fixed the R_5 substituent as CH_2CH_3 and again altered the R group of the 4-alkoxy moiety. When R was CH_3 (**8**), the corresponding IC_{50} value was 0.08 μM, which is equivalent to about 27-fold the potency of **4**, or 3-fold the potency of indomethacin.

Compound **9** ($R = CH_2CH_3$) was slightly less potent than **8**, but still more potent than indomethacin. We then tried systematically replacing the alkyl group with esters of increasing chain length, that is from $-CH_2COOEt$ (**10**) to $-(CH_2)_3COOEt$ (**11**) and to $-(CH_2)_4COOEt$ (**12**). Compound **11**, with an ester substituent of intermediate length, was found to be the least potent one. Amidation of the ester of **10** provided **16** and **17**, which did not show significantly improved activity.

Table 1. The inhibitory effects of compounds **4** and **5** on platelet aggregation induced by thrombin, AA, collagen and PAF (in vitro)[a]

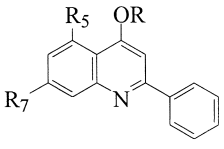


4 , 5

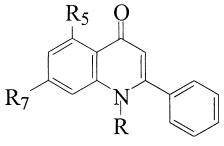
	Compounds (μg/mL)	R	Percent aggregation			
			Thrombin	AA	Collagen	PAF
	Control		92.5±0.5(4)	90.9±0.5(6)	89.2±1.8(4)	92.0±0.8(4)
4	100	CH_3	56.8±8.7(4)***	0.0±0.0(4)***	0.0±0.0(4)***	0.0±0.0(3)***
	50					65.8±4.0(3)***
	20					87.2±0.5(3)***
	5				0.0±0.0(4)***	
	2			0.0±0.0(4)***	9.4±6.0(4)***	
	1			9.3±4.6(4)***	18.5±3.8(4)***	
	0.5			53.4±4.8(4)***	38.6±6.3(4)***	
	0.2			86.0±2.3(4)*	62.8±6.3(4)**	
	0.1			90.8±0.6(4)	85.0±1.3(4)	
	IC_{50}			2.13 μM	1.70 μM	
	Control		92.5±0.5(4)	90.9±0.5(6)	89.2±1.8(4)	92.0±0.8(4)
5	100	C_2H_5	80.0±1.0(3)***	0.0±0.0(5)***	0.0±0.0(4)***	63.8±8.7(4)**
	50				0.0±0.0(4)***	
	20				2.1±1.8(4)***	
	10				5.9±3.2(4)***	
	5			0.0±0.0(5)***	15.9±5.0(4)***	
	2			1.7±1.5(5)***	28.4±8.2(4)***	
	1			4.3±3.8(5)***	36.3±6.3(4)***	
	0.5			23.1±8.9(5)***	43.5±6.4(4)***	
	0.2			86.4±1.5(5)**	78.5±2.7(4)**	
	0.1				85.9±2.1(4)	
	IC_{50}			2.01 μM	3.61 μM	

[a]Platelets were incubated with tested sample or 0.5% DMSO at 37 °C for 1 min, then thrombin (0.1 U/mL), AA (100 μM), collagen (10 μg/mL) or PAF (2 ng/mL) was added to trigger the aggregation. Values are presented as mean±SE, $n = 3–6$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2. The inhibitory effects of compounds **4–17** on platelet aggregation induced by arachidonic acid (in vitro)[a]



4 — 13 , 16 , 17



14 , 15

Compound	R_5	R_7	R	IC_{50} (μM)
4	H	H	CH_3	2.13
5	H	H	C_2H_5	2.01
6	H	H	CH_2COOEt	13.88
7	H	H	CH_2COOH	35.60
8	C_2H_5	H	CH_3	0.08
9	C_2H_5	H	C_2H_5	0.14
10	C_2H_5	H	CH_2COOEt	0.78
11	C_2H_5	H	$(CH_2)_3COOEt$	1.54
12	C_2H_5	H	$(CH_2)_4COOEt$	0.53
13	H	C_2H_5	C_2H_5	3.97
14	H	H	CH_3	218.30
15	C_2H_5	H	CH_3	30.04
16	C_2H_5	H	$CH_2CONHCH_3$	1.88
17	C_2H_5	H	$CH_2CONHC_2H_5$	0.60
Indomethacin				0.25
Aspirin				20.00

[a]Platelets were incubated with a tested sample or 0.5% DMSO at 37 °C for 1 min, then AA (100 μM) was added to trigger aggregation. Aspirin and indomethacin are positive controls. Values are expressed as mean±SE from three to six separations.

The above results indicated that -CH_2CH_3 substitution on R_5 played a key role in enhancing activity (comparing **4–6** with **8–10**). However, repositioning the -CH_2CH_3 substituent from R_5 to R_7 resulted in greatly reduced potency. The activity of **13** was about 1/28 that of **9**. We will continue to explore an explanation for this result.

On the other hand, two 2-phenyl-4-quinolones, **14** and **15**, isomers of **4** and **8**, respectively, with their corresponding R group relocated to their *N*-moiety, demonstrated far weaker activities than their 4-alkoxy counterparts.

In summary, we have prepared 4-alkoxy derivatives of 2-phenylquinolones as well as their related compounds. Investigating the effect of structural modification on antiplatelet activity led to the following preliminary structure–activity relationships.

The 4-alkoxy derivatives (**4–13**, **16**, **17**) demonstrated potent antiplatelet activities, whereas the *N*-alkyl derivatives (**14**, **15**) exhibited much lower activity than their *O*-alkyl isomers. Meanwhile, placing -CH_2CH_3 at R_5 position contributed to greatly enhanced activity.

Among all compounds studied, 5-ethyl-4-methoxy-2-phenylquinoline (**8**), 4-ethoxy-5-ethyl-2-phenylquinoline (**9**), 4-ethoxycarbonylmethoxy-5-ethyl-2-phenylquinoline

(**10**), 4-ethoxycarbonylbutoxy-5-ethyl-2-phenylquinoline (**12**) and 5-ethyl-4-(*N*-ethylcarboxido)methoxy-2-phenylquinoline (**17**) were identified as the five most potent antiplatelet agents, with **8** being the most potent one which had an activity about three times that of indomethacin. The physicochemical properties of phenylquinolines differ widely from those of indomethacin and aspirin, therefore, the action mechanism of **8** was investigated and the preliminary results are described below.

The results indicated that AA- and collagen-induced thromboxane B_2 formation of human platelet from the resting state 0.14 ng/mL to 110.0±27.6 and 11.2±5.3 ng/mL, respectively. At a concentration as low as 0.1 μM, compound **8** decreased the thromboxane B_2 formation to 3.2±0.8 and 3.3±0.6 ng/mL, respectively. Thus, the machanism of antiplatelet action of **8** is possibly through its inhibition on cyclooxygenase or thromboxane synthetase. The exact and detailed mechanism of the antiplatelet action of **8** needs further experiments to elucidate.

Acknowledgements

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