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The evaluation and structure–activity relationships of 2-benzoylaminobenzoic esters and their analogues as anti-inflammatory and anti-platelet aggregation agents

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Abstract—Forty-seven 2-benzoylaminobenzoic esters were synthesized and evaluated in anti-platelet aggregation, inhibition of superoxide anion generation, and inhibition of neutrophil elastase release assays. Most 2-benzoylamino-4-chlorobenzoic acid derivatives showed selective inhibitory effects on arachidonic acid (AA)-induced platelet aggregation. Among them, compounds **6b** and **7b** exhibited more potent inhibitory effects (ca. 200-fold) than aspirin. Additionally, compounds **1a** and **5a** showed strong inhibitory effects on neutrophil superoxide generation with IC_{50} values of 0.65 and 0.17 μM, respectively. However, compounds **6d** and **6e** exhibited dual inhibitory effects on platelet aggregation and neutrophil elastase (NE) release; therefore, these two compounds may be new leads for development as anti-inflammatory and anti-platelet aggregatory agents.

Arterial thromboembotic diseases, for example, acute coronary syndrome and ischemic stroke, which are caused by platelet aggregation, are the major causes of death in developed countries. Antiplatelet drugs, such as aspirin and ticlopidine, are used to protect against myocardial infarction, stroke, cardiovascular death, and other serious vascular events in patients with a history of previous vascular events or known risk factors for cardiovascular disease.[1] However, the current antiplatelet drugs still have some restrictions in their mode of action and efficacy. Therefore, research and development of new generation antiplatelet drugs continue as important targets.

In addition, neutrophils are known to play important roles in a host's defenses against invasion by microorganisms and in the pathogenesis of various diseases such as rheumatoid arthritis, ischemia-reperfusion injury, chronic obstructive pulmonary disease, and asthma.[2] In response to diverse stimuli, activated neutrophils secrete a series of cytotoxins, such as superoxide ($O_2^{\cdot -}$), a precursor of other reactive oxygen species (ROS), granule proteases, and bioactive lipids.[3–6] Thus, suppression of extensive or inappropriate activation of neutrophils using drugs has been proposed as a way to ameliorate inflammatory diseases.

In prior studies, we synthesized 2-phenyl substituted quinazolines and 2,8-disubstituted benzoxazinones that exhibited significant inhibitory effects on platelet aggregation and superoxide ($O_2^{\cdot -}$) generation of neutrophils.[7,8] Among them, 2-(2-bromophenyl)-8-methoxybenzoxazinone showed concentration-dependent inhibitory effects on platelet aggregation, ATP release, P-selectin expression, and intracellular calcium mobilization caused by thrombin. In contrast, it had no significant effect on either SFLLRN- or GYPGKF-induced platelet aggregation.[9] In our continuing research on anti-platelet aggregation and anti-inflammatory agents, we also found that certain 2-benzoylaminobenzoic acid derivatives exhibited higher anti-platelet aggregation and anti-inflammatory potency than related 2-phenylbenzoxazinones.

Keywords: 2-Benzoylaminobenzoic esters; Anti-inflammatory; Anti-platelet aggregation agents.
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Previously, 2-benzoylaminobenzoic acid derivatives, such as phytoalexin, were isolated from *Dianthus* species of the family Caryophyllaceae, while these plants were infected by *Phytophthora parasitica*.[10] Therefore, we synthesized 2-benzoylaminobenzoic acid derivatives and investigated their anti-fungal effects.[11–13] Additionally, such compounds were also reported recently as anti-proliferative[14] and anti-hypercholesterolemic[15] agents. In the present study, we report the preparation, preliminary pharmacological data, and structure-activity relationships of new 2-benzoylaminobenzoic acid derivatives.

Forty-seven 2-benzoylaminobenzoic acid derivatives (**1a**–**1o**, **2a**–**2e**, **3a**–**3e**, **3k**–**3o**, **4a**–**4e**, **5a**, **6a**–**6e**, **7a**–**7e**, and **8a**) were synthesized (Schemes 1 and 2, Tables 1 and 2). All new products were fully characterized using spectroscopic data as shown in Table 3.[16,17]

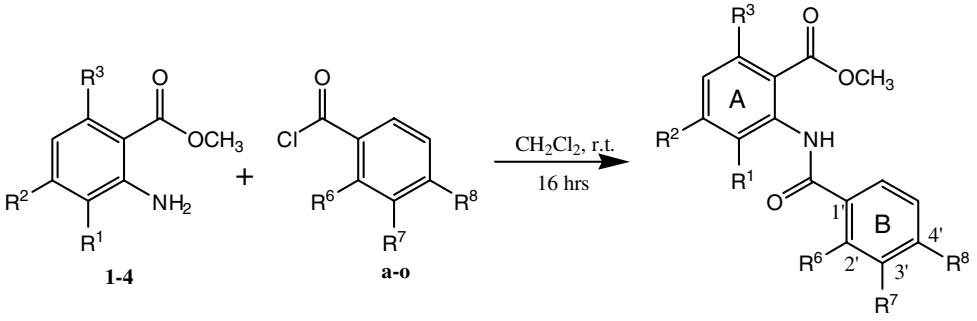
As previously reported,[7,8] benzoxazinones exhibited selective inhibitory effects on platelet aggregation induced by arachidonic acid (AA) and thrombin (Thr). Thus, these two inducers were used in the present work. Aspirin and indomethacin were also assayed as positive controls.[18] Bioassay results are shown in Table 4.

Most 2-benzoylamino-4-chlorobenzoic acid derivatives showed selective inhibitory effects on AA-induced platelet aggregation. Among them, compounds **6b** and **7b** exhibited the highest potency, about 200 times stronger than aspirin. Furthermore, all 2-benzoylamino-3-chloro- and 2-benzoylamino-6-chlorobenzoic acid derivatives were not active or only weakly active.

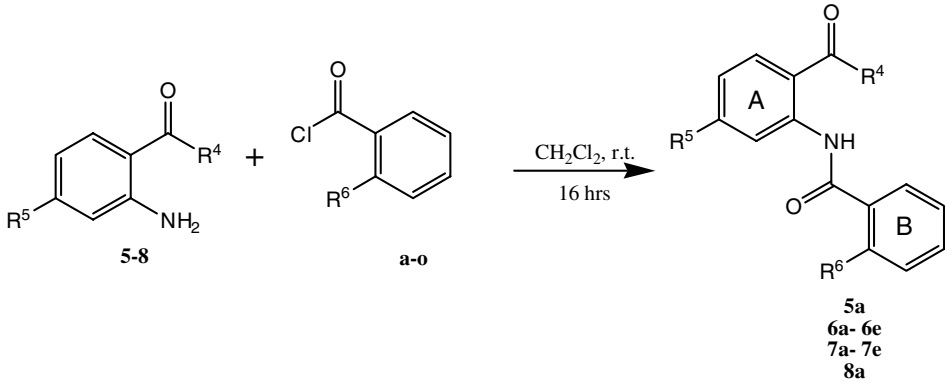
On the basis of these results, several structure–activity relationship conclusions for anti-platelet aggregation activity were summarized as follows:

1. 2-Benzoylamino-4-chlorobenzoic acid derivatives showed selective inhibitory effects on AA-induced platelet aggregation.
2. Bioactivity was essentially abolished in the presence of 3- or 6-chloro substitution.
3. 2-(2′-Chlorobenzoylamino)-4-chlorobenzoic acid analogues exhibited more potent effects than those with fluoro, bromo, methoxy, or methyl groups.
4. Among compounds **1a**–**1e**, **6a**–**6e**, and **7a**–**7e**, the bioactivity generally improved by increasing the ester chain length.

Neutrophil elastase release and neutrophil superoxide generation induced by *N*-formyl-L-methionyl-L-lucyl-L-



Scheme 1.



Scheme 2.

Table 1. The 2-benzoylamino-benzoic acid derivates

Compound	R^1	R^2	R^3	R^6	R^7	R^8
1a	H	H	H	F	H	H
1b	H	H	H	Cl	H	H
1c	H	H	H	Br	H	H
1d	H	H	H	CH_3	H	H
1e	H	H	H	OCH_3	H	H
1f	H	H	H	H	F	H
1g	H	H	H	H	Cl	H
1h	H	H	H	H	Br	H
1i	H	H	H	H	CH_3	H
1j	H	H	H	H	OCH_3	H
1k	H	H	H	H	H	F
1l	H	H	H	H	H	Cl
1m	H	H	H	H	H	Br
1n	H	H	H	H	H	CH_3
1o	H	H	H	H	H	OCH_3
2a	Cl	H	H	F	H	H
2b	Cl	H	H	Cl	H	H
2c	Cl	H	H	Br	H	H
2d	Cl	H	H	CH_3	H	H
2e	Cl	H	H	OCH_3	H	H
3a	H	Cl	H	F	H	H
3b	H	Cl	H	Cl	H	H
3c	H	Cl	H	Br	H	H
3d	H	Cl	H	CH_3	H	H
3e	H	Cl	H	OCH_3	H	H
3k	H	Cl	H	H	H	F
3l	H	Cl	H	H	H	Cl
3m	H	Cl	H	H	H	Br
3n	H	Cl	H	H	H	CH_3
3o	H	Cl	H	H	H	OCH_3
4a	H	H	Cl	F	H	H
4b	H	H	Cl	Cl	H	H
4c	H	H	Cl	Br	H	H
4d	H	H	Cl	CH_3	H	H
4e	H	H	Cl	OCH_3	H	H

Table 2. The 2-benzoylamino-benzoic acid derivates

Compound	R^4	R^5	R^6
5a	OCH_2CH_3	H	F
6a	OCH_2CH_3	Cl	F
6b	OCH_2CH_3	Cl	Cl
6c	OCH_2CH_3	Cl	Br
6d	OCH_2CH_3	Cl	CH_3
6e	OCH_2CH_3	Cl	OCH_3
7a	$OCH_2CH_2CH_3$	Cl	F
7b	$OCH_2CH_2CH_3$	Cl	Cl
7c	$OCH_2CH_2CH_3$	Cl	Br
7d	$OCH_2CH_2CH_3$	Cl	CH_3
7e	$OCH_2CH_2CH_3$	Cl	OCH_3
8a	CH_3	H	F

phenylaline (fMLP) were also tested as a measure of anti-inflammatory activity.[19] Results are presented in Table 3. Comparison of series **1a**–**1e**, **2a**–**2e**, **3a**–**3e**, and **4a**–**4e** showed that only series **1a**–**1e** exhibited inhibitory effects on neutrophil $O_2^{\cdot-}$ generation, indicating that substitution of a chlorine atom on A ring resulted in decreased bioactivity. Among this series, compounds **1a** and **5a** showed strong inhibitory effects on neutrophil $O_2^{\cdot-}$ generation. The results indicated that when the methyl ester was replaced by an ethyl ester, the inhibitory effect on neutrophil $O_2^{\cdot-}$ generation was increased. Moreover, compounds **1h**, **1i**, **5a**, **6d**, and **6e** also inhibited neutrophil elastase (NE) release induced by fMLP. According to these results, the following structure–activity relationships for anti-inflammatory effects were proposed.

1. Chloro-substitution at position C-3, -4, or -6 in A ring resulted in decreased inhibitory effects on neutrophil $O_2^{\cdot-}$ generation.
2. Bioactivity was increased by replacing the methyl ester with an ethyl ester. These results suggested that the hydrophobicity of 2-benzoylamino-benzoic acid derivatives plays an important role in the anti-inflammatory effect. On the other hand, when the ester group was replaced by a ketone group (**8a**), the anti-inflammatory activity decreased.
3. Anti-inflammatory activity depended dramatically upon the identity and position of B ring substituents. For inhibition of neutrophil $O_2^{\cdot-}$ generation, a *meta*-fluoro substituent appeared to be the most favorable. However, the best choice for inhibition of NE release was a *meta*-methyl group. Furthermore, for inhibition of neutrophil $O_2^{\cdot-}$ generation, C-2′ substitution resulted in higher potency than C-3′ or C-4′ substitution, but C-3′ substitution was preferable in the NE release inhibition assay.

In conclusion, the bioassay results of these compounds show dramatic relationships between structure and anti-platelet aggregation and anti-inflammation effects. This is the first reported evaluation of the anti-platelet aggregatory and anti-inflammatory effects of 2-benzoylaminobenzoic acid derivatives. In contrast to the anti-platelet aggregation of bezoxazinones, the 2-benzoylamino-4-chlorobenzoic acid derivatives showed selective inhibitory effects on AA-induced platelet aggregation. These observations confirmed that the oxazinone ring is necessary for inhibiting platelet aggregation induced by thrombin.[8] Among the tested compounds, **6b** and **7b** exhibited 200-fold more potent inhibitory effects than aspirin on AA-induced platelet aggregation, which suggested that 2-benzoylaminobenzoic acid derivatives also can be designed as aspirin-like anti-platelet agents. Additionally, compounds **1a** and **5a** showed strong inhibitory effects on neutrophil superoxide generation with IC_{50} values of 0.65 and 0.17 μM, respectively. The present results led to the identification of **1a** and **5a** as a new generic class of lead compounds for anti-inflammatory agents. Additionally, compounds **6d** and **6e** exhibited dual inhibitory effects on platelet aggregation and NE release; therefore, these two compounds may represent new leads for development as anti-inflammatory and anti-platelet aggregatory agents.

Table 3. The ^{1}H NMR data (in $CDCl_3$) of new compounds (δ)

Compound	δ_H (mult., J)
2a	9.43 (1H, br d, J = 13.6 Hz, NH), 8.17 (1H, t, J = 8.0 Hz, Ar-H), 7.83 (1H, d, J = 8.0 Hz, Ar-H), 7.64 (1H, d, J = 8.0 Hz, Ar-H), 7.54 (1H, br q, J = 8.0 Hz, Ar-H), 7.25 (3H, m, Ar-H), 3.87 (3H, s, COOMe)
2b	8.88 (1H, br s, NH), 7.81 (1H, br t, J = 8.0 Hz, Ar-H), 7.63 (1H, d, J = 8.0 Hz, Ar-H), 7.42 (3H, m, Ar-H), 7.25 (1H, br m, Ar-H), 3.89 (3H, s, COOMe)
2c	8.72 (1H, br s, NH), 7.83 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.72 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.65 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.64 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.41 (1H, td, J = 8.0, 1.6 Hz, Ar-H), 7.32 (1H, td, J = 8.0, 1.6 Hz, Ar-H), 7.26 (1H, t, J = 8.0 Hz, Ar-H), 3.89 (3H, s, COOMe)
2d	8.65 (1H, br s, NH), 7.84 (1H, dd, J = 7.6, 1.6 Hz, Ar-H), 7.68 (1H, d, J = 7.6 Hz, Ar-H), 7.63 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.37 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.27 (2H, br t, J = 8.0 Hz, Ar-H), 7.25 (1H, t, J = 7.6 Hz, Ar-H), 3.89 (3H, s, COOMe), 2.55 (3H, s, Ar-CH_3)
2e	10.52 (1H, br s, NH), 8.26 (1H, dd, J = 7.6, 1.6 Hz, Ar-H), 7.74 (1H, dd, J = 7.6, 1.6 Hz, Ar-H), 7.59 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.51 (1H, m, Ar-H), 7.20 (1H, t, J = 8.0 Hz, Ar-H), 7.11 (1H, td, J = 8.0, 1.6 Hz, Ar-H), 7.05 (1H, t, J = 7.6 Hz, Ar-H), 4.08 (3H, s, OMe), 3.84 (3H, s, COOMe)
3a	11.90 (1H, br d, J = 7.6 Hz, NH), 9.01 (1H, d, J = 2.0 Hz, Ar-H), 8.06 (1H, td, J = 7.6, 2.0 Hz, Ar-H), 7.99 (1H, d, J = 8.0 Hz, Ar-H), 7.52 (1H, m, Ar-H), 7.30 (1H, t, J = 8.0 Hz, Ar-H), 7.20 (1H, dd, J = 8.0, 8.0 Hz, Ar-H), 7.10 (1H, dd, J = 8.0, 2.0 Hz, Ar-H), 3.94 (3H, s, COOMe)
3c	11.52 (1H, br s, NH), 9.00 (1H, d, J = 2.0 Hz, Ar-H), 7.99 (1H, d, J = 8.0 Hz, Ar-H), 7.66 (1H, dd, J = 8.0, 2.0 Hz, Ar-H), 7.59 (1H, dd, J = 8.0, 2.0 Hz, Ar-H), 7.42 (1H, td, J = 8.0, 2.0 Hz, Ar-H), 7.33 (1H, td, J = 8.0, 2.0 Hz, Ar-H), 7.12 (1H, dd, J = 8.0, 2.0 Hz, Ar-H), 3.89 (3H, s, COOMe)
3l	12.09 (1H, br s, NH), 8.99 (1H, d, J = 2.0 Hz, Ar-H), 7.99 (1H, d, J = 8.4 Hz, Ar-H), 7.96 (2H, d, J = 8.8 Hz, Ar-H), 7.49 (2H, d, J = 8.8 Hz, Ar-H), 7.09 (1H, dd, J = 8.4, 2.0 Hz, Ar-H), 3.96 (3H, s, COOMe)
3m	12.08 (1H, br s, NH), 8.97 (1H, d, J = 2.0 Hz, Ar-H), 7.97 (1H, d, J = 8.4 Hz, Ar-H), 7.87 (2H, d, J = 8.8 Hz, Ar-H), 7.64 (2H, d, J = 8.8 Hz, Ar-H), 7.08 (1H, dd, J = 8.4, 2.0 Hz, Ar-H), 3.95 (3H, s, COOMe)
3n	11.98 (1H, br s, NH), 8.99 (1H, d, J = 2.4 Hz, Ar-H), 7.92 (1H, d, J = 8.8 Hz, Ar-H), 7.88 (2H, d, J = 8.4 Hz, Ar-H), 7.27 (2H, d, J = 8.4 Hz, Ar-H), 7.01 (1H, dd, J = 8.8, 2.4 Hz, Ar-H), 3.95 (3H, s, COOMe), 2.39 (3H, s, Ar-CH_3)
3o	12.00 (1H, br s, NH), 9.03 (1H, d, J = 2.0 Hz, Ar-H), 8.00 (2H, d, J = 8.4 Hz, Ar-H), 7.99 (1H, d, J = 8.8 Hz, Ar-H), 7.06 (1H, dd, J = 8.8, 2.0 Hz, Ar-H), 7.01 (2H, d, J = 8.4 Hz, Ar-H), 3.96 (3H, s, COOMe), 3.88 (3H, s, Ar-CH_3)
4a	9.76 (1H, br d, J = 13.2 Hz, NH), 8.36 (1H, d, J = 8.0 Hz, Ar-H), 8.13 (1H, td, J = 8.0, 1.6 Hz, Ar-H), 7.53 (1H, m, Ar-H), 7.39 (1H, d, J = 8.0 Hz, Ar-H), 7.30 (1H, td, J = 8.0, 1.6 Hz, Ar-H), 7.22 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.19 (1H, dd, J = 8.0, 8.0 Hz, Ar-H), 4.00 (3H, s, COOMe)
4b	9.41 (1H, br s, NH), 8.41 (1H, d, J = 8.0 Hz, Ar-H), 7.70 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.41 (4H, m, Ar-H), 7.24 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 3.96 (3H, s, COOMe)
4c	9.30 (1H, br s, NH), 8.43 (1H, d, J = 8.0 Hz, Ar-H), 7.65 (1H, dd, J = 8.0, 1.2 Hz, Ar-H), 7.59 (1H, dd, J = 8.0, 1.2 Hz, Ar-H), 7.43 (2H, m, Ar-H), 7.34 (1H, td, J = 8.0, 1.2 Hz, Ar-H), 7.24 (1H, dd, J = 8.0, 1.2 Hz, Ar-H), 3.96 (3H, s, COOMe)
4d	9.37 (1H, br s, NH), 8.45 (1H, d, J = 8.0 Hz, Ar-H), 7.52 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.39 (2H, m, Ar-H), 7.29 (2H, br t, J = 8.0 Hz, Ar-H), 7.22 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 3.96 (3H, s, COOMe), 2.54 (3H, s, Ar-CH_3)
4e	10.65 (1H, br s, NH), 8.39 (1H, d, J = 8.0 Hz, Ar-H), 8.27 (1H, dd, J = 8.0, 2.0 Hz, Ar-H), 7.51 (1H, ddd, J = 8.4, 8.0, 2.0 Hz, Ar-H), 7.38 (1H, t, J = 8.0 Hz, Ar-H), 7.17 (1H, br t, J = 8.0 Hz, Ar-H), 7.11 (1H, br t, J = 8.0 Hz, Ar-H), 7.03 (1H, d, J = 8.0 Hz, Ar-H), 4.10 (3H, s, OMe), 3.96 (3H, s, COOMe)
6a	11.95 (1H, br d, J = 7.6, NH), 9.02 (1H, d, J = 2.2 Hz, Ar-H), 8.06 (1H, td, J = 8.0, 1.8 Hz, Ar-H), 8.02 (1H, d, J = 8.6 Ar-H), 7.53 (1H, m, Ar-H), 7.29 (1H, td, J = 8.0, 7.0, 1.2 Hz, Ar-H), 7.20 (1H, ddd, J = 11.6, 7.0,1.2 Hz, Ar-H), 7.11 (1H, dd, J = 8.6, 2.2 Hz, Ar-H), 4.40 (2H, q, J = 7.2 Hz, OCH_2CH$_3$), 1.41 (3H, t, J = 7.2 Hz, OCH$_2$CH_3)
6b	11.47 (1H, s, NH), 8.82 (1H, d, J = 1.8 Hz, Ar-H), 7.82 (1H, d, J = 8.4 Hz, Ar-H), 7.47 (1H, dd, J = 7.6, 2.6, Ar-H), 7.23 (3H, m, Ar-H), 6.91 (1H, dd, J = 8.4, 1.8 Hz, Ar-H), 4.16 (2H, q, J = 7.2 Hz, OCH_2CH$_3$), 1.92 (3H, t, J = 7.2 Hz, OCH$_2$CH_3)
6c	11.55 (1H, s, NH), 9.00 (1H, d, J = 2.2 Hz, Ar-H), 8.02 (1H, d, J = 8.8 Hz, Ar-H), 7.66 (1H, dd, J = 7.6, 1.0 Hz Ar-H), 7.59 (1H, dd, J = 7.2, 1.8 Hz, Ar-H), 7.43 (1H, m, Ar-H), 7.35 (1H, m, Ar-H), 7.13 (1H, dd, J = 8.8, 2.2 Hz, Ar-H), 4.35 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 1.38 (3H, t, J = 7.0 Hz, OCH$_2$CH_3)
6d	11.56 (1H, s, NH), 9.03 (1H, d, J = 2.2 Hz, Ar-H), 8.01 (1H, d, J = 8.8 Hz, Ar-H), 7.60 (1H, d, J = 7.6 Hz, Ar-H), 7.36 (3H, m, Ar-H), 7.10 (1H, dd, J = 8.8, 2.2 Hz, Ar-H), 4.36 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 2.56 (3H, s, Ar-CH_3), 1.39 (3H, t, J = 7.0 Hz, OCH$_2$CH_3)
6e	12.30 (1H, s, NH), 9.09 (1H, d, J = 1.8 Hz, Ar-H), 8.18 (1H, dd, J = 7.6, 1.8 Hz, Ar-H), 7.98 (1H, d, J = 8.8 Hz, Ar-H), 7.49 (1H,, ddd, J = 8.4, 7.6,1.8 Hz, Ar-H), 7.06 (3H, m, Ar-H), 7.10 (1H, dd, J = 8.8, 1.8 Hz, Ar-H), 4.38 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 4.06 (3H, s, OMe), 1.41 (3H, t, J = 7.0 Hz, OCH$_2$CH_3)
7a	11.95 (1H, br d, J = 7.6 Hz, NH), 9.03 (1H, d, J = 2.2 Hz, Ar-H), 8.06 (1H, td, J = 7.8, 2.0 Hz, Ar-H), 8.02 (1H, d, J = 8.6 Hz, Ar-H), 7.53 (1H, m, Ar-H), 7.31 (1H, td, J = 7.8, 1.0 Hz, Ar-H), 7.20 (1H, ddd, J = 8.6, 7.8, 1.0 Hz, Ar-H), 7.11 (1H, dd, J = 8.6, 2.2 Hz, Ar-H), 4.30 (2H, t, J = 6.8 Hz, OCH_2CH$_2$CH$_3$), 1.80 (2H, m, OCH$_2$CH_2CH$_3$), 1.03 (3H, t, J = 7.2 Hz, OCH$_2$CH$_2$CH_3)
7b	11.63 (1H, s, NH), 9.01 (1H, d, J = 2.2 Hz, Ar-H), 8.01 (1H, d, J = 8.6 Hz, Ar-H), 7.64 (1H, dd, J = 6.6, 2.8 Hz, Ar-H), 7.41 (3H, m, Ar-H), 7.12 (1H, d, J = 8.6, 2.2 Hz, Ar-H), 4.25 (2H, t, J = 6.0 Hz, OCH_2CH$_2$CH$_3$), 1.78 (2H, m, OCH$_2$ CH_2CH$_3$), 1.00 (3H, t, J = 7.4 Hz, OCH$_2$CH$_2$CH_3)
7c	11.56 (1H, s, NH), 9.00 (1H, d, J = 2.2 Hz, Ar-H), 8.01 (1H, d, J = 8.6 Hz, Ar-H), 7.61 (1H, dd, J = 8.0, 1.6 Hz, Ar-H), 7.47 (1H, dd, J = 7.4, 2.2 Hz, Ar-H), 7.42 (1H, m, Ar-H), 7.34 (1H, m, Ar-H), 7.12 (1H, dd, J = 8.6, 2.2 Hz, Ar-H), 4.25 (2H, t, J = 6.0 Hz, OCH_2CH$_2$CH$_3$), 1.78 (2H, m, OCH$_2$CH_2CH$_3$), 1.00 (3H, t, J = 7.4 Hz, OCH$_2$CH$_2$CH_3)

Table 3 (*continued*)

Compound	δ_H (mult., *J*)
7d	11.68 (1H, s, NH), 9.03 (1H, d, *J* = 2.0 Hz, Ar-H), 8.00 (1H, d, *J* = 8.8 Hz, Ar-H), 7.61 (1H, d, *J* = 7.2 Hz, Ar-H), 7.36 (3H, m, Ar-H), 7.10 (1H, dd, *J* = 8.8, 2.0 Hz, Ar-H), 4.25 (2H, t, *J* = 6.6 Hz, $OCH_2CH_2CH_3$), 2.56 (3H, s, Ar-CH_3), 1.78 (2H, m, $OCH_2CH_2CH_3$), 1.01 (3H, t, *J* = 7.4 Hz, $OCH_2CH_2CH_3$)
7e	12.25 (1H, s, NH), 9.09 (1H, d, *J* = 2.2 Hz, Ar-H), 8.17 (1H, dd, *J* = 8.4, 1.8 Hz, Ar-H), 7.98 (1H, d, *J* = 8.4 Hz, Ar-H), 7.48 (1H,, ddd, *J* = 8.4, 7.6, 1.8, Ar-H), 7.06 (3H, m, Ar-H), 7.10 (1H, dd, *J* = 8.8, 2.2 Hz, Ar-H), 4.28 (2H, t, *J* = 6.6 Hz, $OCH_2CH_2CH_3$), 4.06 (3H, s, OMe), 1.80 (2H, m, $OCH_2CH_2CH_3$), 1.03 (3H, t, *J* = 7.2 Hz, $OCH_2CH_2CH_3$)

Table 4. The bioactivity data of compounds (μM)

Compound	Antiplatelet aggregation		Anti-inflammation	
	AA	Thr	$O_2^{\cdot-}$ generation	NE release
Aspirin[c]	151.6 ± 1.2[a,b]	>100	>40	>40
Indomethacin[c]	0.14 ± 0.0	>100	>40	>40
DPI[d]			0.86 ± 0.35	
PMSF[d]				130.93 ± 29.10
1a	10.8 ± 3.7	>100	0.65 ± 0.36	>40
1b	11.0 ± 3.4	>100	11.01 ± 2.82	>40
1c	22.9 ± 6.2	>100	12.02 ± 2.10	>40
1d	16.6 ± 7.3	>100	9.26 ± 2.22	>40
1e	8.0 ± 2.0	>100	7.41 ± 0.59	>40
1f	>100	>100	10.56 ± 5.75	>40
1g	>100	>100	>40	>40
1h	>100	>100	>40	15.40 ± 2.14
1i	>100	>100	>40	18.30 ± 0.76
1j	>100	>100	>40	>40
1k	>100	>100	>40	>40
1l	>100	>100	>40	>40
1m	>100	>100	>40	>40
1n	>100	>100	>40	>40
1o	>100	>100	>40	>40
2a	>100	>100	>40	>40
2b	>100	>100	>40	>40
2c	>100	>100	>40	>40
2d	>100	>100	>40	>40
2e	66.6 ± 23.6	>100	>40	>40
3a	2.2 ± 0.5	>100	>40	>40
3b	1.1 ± 0.3	>100	>40	>40
3c	2.6 ± 0.2	>100	>40	>40
3d	4.3 ± 2.5	>100	>40	>40
3e	2.2 ± 0.6	>100	>40	>40
3k	>100	>100	>40	>40
3l	>100	>100	>40	>40
3m	>100	>100	>40	>40
3n	>100	>100	>40	>40
3o	>100	>100	>40	>40
4a	78.2 ± 15.4	>100	>40	>40
4b	80.8 ± 13.6	>100	>40	>40
4c	76.4 ± 16.7	>100	>40	>40
4d	>100	>100	>40	>40
4e	62.3 ± 15.4	>100	>40	>40
5a	NT	>100	0.17 ± 0.03	14.35 ± 1.92
6a	3.8 ± 1.1	>100	>40	>40
6b	0.8 ± 0.0	>100	>40	>40
6c	3.6 ± 0.0	>100	>40	>40
6d	4.9 ± 1.1	>100	>40	2.83 ± 1.05
6e	4.9 ± 1.1	>100	>40	4.31 ± 1.55
7a	1.6 ± 0.0	>100	>40	>40
7b	0.7 ± 0.0	>100	>40	>40
7c	3.5 ± 0.0	>100	>40	>40
7d	2.2 ± 0.5	>100	>40	>40
7e	23.4 ± 6.2	>100	>40	>40
8a	NT	>100	24.1 ± 2.8	>40

[a] Platelets were pre-incubated with DMSO (0.5%, control), aspirin or test compound at 37 °C for 3 min before addition of the inducer.
[b] The IC_{50} values are presented as means ± SEM ($n = 3$).
[c] Aspirin and indomethacin, two cyclooxygenase inhibitors, were used as positive control in platelet aggregation assay.
[d] Diphenyleneiodonium (DPI) and phenylmethylsulfonyl fluoride (PMSF) were used as positive control in anti-inflammatory assay.

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References and notes

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16. General experimental procedure for the synthesis of compounds **1a**–**1o**, **2a**–**2e**, **3a**–**3e**, **3k**–**3o**, and **4a**–**4e**. To a DCM solution of methyl anthranilate (1.0 mmol) were added corresponding substituted benzoyl chlorides. The reaction mixture was stirred overnight at room temperature for 16 h, respectively. The solvent was evaporated at reduced pressure. The residue was purified by column chromatography (Si-Gel) using $CHCl_3$/hexane (2:7) mixture to afford the products. Compound **2a**: EI-MS *m*/*z*: 157 (100), 274 (42), 306 $[M]^+$ (38). Compound **2b**: EI-MS *m*/*z*: 139 (100), 256 (40), 324 $[M]^+$ (1). Compound **2c**: EI-MS *m*/*z*: 183 (100), 332 (50), 368 $[M]^+$ (0.5). Compound **2d**: EI-MS *m*/*z*: 91 (100), 119 (88), 268 (52), 304 $[M]^+$ (0.2). Compound **2e**: EI-MS *m*/*z*: 135 (100), 284 (54), 320 $[M]^+$ (1). Compound **3a**: EI-MS *m*/*z*: 307 (18), 309 $[M]^+$ (6). Compound **3c**: EI-MS *m*/*z*: 367 $[M]^+$ (20), 369 (27). Compound **3e**: EI-MS *m*/*z*: 319 $[M]^+$ (3). Compound **3l**: EI-MS *m*/*z*: 323 $[M]^+$ (15), 325 (10). Compound **3m**: EI-MS *m*/*z*: 367 $[M]^+$ (21), 369 (28). Compound **3n**: EI-MS *m*/*z*: 303 $[M]^+$ (18). Compound **3o**: EI-MS *m*/*z*: 319 $[M]^+$ (7). Compound **4a**: EI-MS *m*/*z*: 123 (100), 248 (40), 307 $[M]^+$ (7). Compound **4b**: EI-MS *m*/*z*: 139 (100), 264 (28), 323 $[M]^+$ (5). Compound **4c**: EI-MS *m*/*z*: 183 (100), 310 (31), 367 $[M]^+$ (7), 369 (8). Compound **4d**: EI-MS *m*/*z*: 91 (100), 119 (99), 244 (17), 303 $[M]^+$ (2). Compound **4e**: EI-MS *m*/*z*: 135 (100), 260 (17), 319 $[M]^+$ (4).
17. General experimental procedure for the synthesis of compounds **5a**, **6a**–**6e**, **7a**–**7e**, and **8a**. To a DCM solution of ethyl anthranilate, ethyl 2-amino-4-chloro-benzoate, propyl 2-amino-4-chloro-benzoate, or 2-amino-acetophenone (each 1.0 mmol) was added, respectively, and then mixed with corresponding substituted benzoyl chlorides. The reaction mixture was stirred overnight at room temperature for 16 h, respectively. The solvent was evaporated at reduced pressure. The residue was purified by column chromatography (Si-Gel) using $CHCl_3$/hexane (1:4) mixture to afford the products. Compound **6a**: EI-MS *m*/*z*: 123 (100), 248 (75), 250 (22), 321 $[M]^+$ (35), 323(12). Compound **6b**: EI-MS *m*/*z*: 139 (100), 141 (33), 264 (34), 266 (23), 320 (99), 322 (65), 337$[M]^+$ (60). Compound **6c**: EI-MS *m*/*z*: 183 (100), 185 (99), 230 (55), 381 $[M]^+$ (15), 383 (20). Compound **6d**: EI-MS *m*/*z*: 119 (100), 300 (31), 317$[M]^+$ (12). Compound **6e**: EI-MS *m*/*z*: 135 (100), 201 (16), 333 $[M]^+$ (22), 334 (15). Compound **7a**: EI-MS *m*/*z*: 123 (100), 248 (65), 318 (49), 335 $[M]^+$ (32). Compound **7b**: EI-MS *m*/*z*: 139 (100), 264 (22), 351 $[M]^+$ (13), 353 (9). Compound **7c**: EI-MS *m*/*z*: 185(100), 230 (44), 395 $[M]^+$ (7), 397 (8). Compound **7d**: EI-MS *m*/*z*: 119 (100), 331 $[M]^+$ (5). Compound **7e**: EI-MS *m*/*z*: 135 (100), 213 (79), 347 $[M]^+$ (2).
18. Antiplatelet aggregation assays: see Wu, C. C.; Wang, W. Y.; Kuo, R. Y.; Chang, F. R.; Wu, Y. C. *Eur. J. Pharmacol.* **2004**, *483*, 187.
19. Superoxide anion formation and elastase release: see Hwang, T. L.; Hung, H. S.; Kao, S. H.; Teng, C. M.; Wu, C. C.; Cheng, S. J. S. *Mol. Pharmacol.* **2003**, *64*, 1.