

The evaluation of 2,8-disubstituted benzoxazinone derivatives as anti-inflammatory and anti-platelet aggregation agents

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Abstract—A series of 2,8-disubstituted benzoxazinones were synthesized and subjected to anti-platelet aggregation, inhibition of superoxide anion generation, and inhibition of neutrophil elastase release assays. Among them, 2-(2'-substituted-phenyl)-benzoxazinones exhibited significant inhibitory effect to target assays. Additionally, all of them were more potent than aspirin on AA-induced platelet aggregation, and these suggested that 2-(2'-substituted-phenyl)-benzoxazinones also possess aspirin-like activity. On the other hand, the compounds **6** and **16** showed inhibitory effects on neutrophil elastase release and superoxide generation.

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1. Introduction

Human neutrophils play an important role 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.^{1–5} In response to diverse stimuli, activated neutrophils secrete a series of haphazard cytotoxins, such as superoxide anion ($O_2^{\cdot-}$), a precursor of other reactive oxygen species (ROS), granule proteases, and bioactive lipids.^{2,6,7} The agents exhibited the inhibitory effect on the neutrophils activation has been proposed to ameliorate these inflammatory diseases. Furthermore, neutrophil elastase (NE) is a major secreted product of activated neutrophils and a major contributor to destruction of tissue in chronic inflammatory disease. Therefore, NE appears to be a target for therapy of chronic inflammatory diseases.⁸

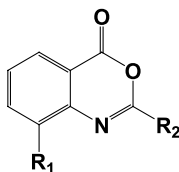
On the other hand, numerous evidence indicate that platelets contribute significantly to the pathogenesis of arterial thromboembolic diseases, such as acute coronary syndrome, and ischemic stroke, which are the major causes of death in developed countries.^{9–11} However,

current anti-platelet drugs still have considerable limitation in their mode of action and efficacy. Therefore, in continuing our research on anti-platelet aggregation and anti-inflammatory agents from natural sources or their derivatives, we found the 2-phenyl-benzoxazinones, exhibited both anti-platelet aggregation and anti-inflammatory effects.

2-Substituted benzoxazinones were as mechanism-based inhibitors of standard serine proteases of the chymotrypsin superfamily^{12,13} and inhibit by formation of an acyl-enzyme complex through attack of the active site serine on the carbonyl group.^{14,15} Therefore, the 2-substituted benzoxazinones showed bioactivities on human leukocyte elastase,^{13,15–17} C1r serine protease of the complement system,^{18,19} cathepsin G,¹⁷ human chymase,¹⁷ tissue factor VIIa,²⁰ anti-HCoVs, and ICAM-1 inhibitors.²¹ Additionally, we synthesized a series 2-phenyl substituted quinazolines, which exhibited significant activities to platelet aggregation and superoxide ($O_2^{\cdot-}$) generation of neutrophils recently,²² the structures were similar to benzoxazinones. Furthermore, the foregoing benzoxazinone derivatives were almost modified on the C-5, 6, or 7 positions, and no one focus on the modification of C-8.²³ Therefore, a series of 2,8-substituted benzoxazinones were synthesized. In here, we report the preparation, preliminary pharmacological data, and structure activities relationship of 2,8-disubstituted benzoxazinones derivatives (Table 1) as potent anti-platelet aggregation, and anti-inflammatory agents.

Keywords: Benzoxazinones; Anti-platelet aggregation; Neutrophil elastase; Superoxide generation.

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Table 1. The compounds with benzoxazinone skeleton and their bioactivities

R ₁	R ₂	Anti-platelet aggregation IC ₅₀ (μM) ^{a,b}				Anti-inflammatory IC ₅₀ (μM)	
		AA (100 μM)	Col (10 μg/ml)	Thr (0.1 U/ml)	PAF (2 ng/ml)	O ₂ ^{•-} generation	NE release
Aspirin ^c		34.6 ± 1.0	34.9 ± 0.1	>100	>100		
DPI ^d						0.46 ± 0.21	
1	OCH ₃ 4'-Bromo-phenyl	>100	>100	>100	>100	>40	NT
2	OCH ₃ 4'-Chloro-phenyl	>100	>100	>100	>100	>40	NT
3	OCH ₃ 2'-Bromo-phenyl	13.0 ± 0.8 ^a	21.1 ± 4.0	4.4 ± 1.4	>100	16.67 ± 4.38	NT
4	OCH ₃ 2'-Chloro-phenyl	11.8 ± 1.9	35.5 ± 4.5	6.9 ± 0.7	>100	14.67 ± 5.01	NT
5	OCH ₃ 2'-Fluoro-phenyl	8.0 ± 2.0	39.4 ± 7.1	7.2 ± 2.8	>100	9.74 ± 1.40	NT
6	OCH ₃ 2'-Methoxy-phenyl	>100	>100	>100	>100	13.99 ± 1.55	1.59 ± 0.11
7	OCH ₃ 2'-Methyl-phenyl	8.4 ± 2.2	34.3 ± 2.9	67.0 ± 2.9	>100	11.39 ± 0.56	10.56 ± 3.03
8	Cl 2'-Bromo-phenyl	10.2 ± 2.4	47.0 ± 5.9	7.1 ± 0.5	87.5 ± 10.2	6.56 ± 3.09	1.69 ± 0.30
9	Cl 2'-Chloro-phenyl	6.1 ± 2.3	30.0 ± 4.3	6.6 ± 0.3	69.5 ± 3.1	8.66 ± 1.44	1.89 ± 1.10
10	Cl 2'-Fluoro-phenyl	12.7 ± 2.3	49.1 ± 13.9	14.7 ± 1.0	87.4 ± 7.2	>40	6.03 ± 0.65
11	Cl 2'-Methoxy-phenyl	25.4 ± 5.2	44.9 ± 11.3	14.8 ± 2.3	70.3 ± 6.9	10.03 ± 0.59	1.91 ± 0.14
12	Cl 2'-Methyl-phenyl	13.8 ± 3.9	63.3 ± 13.6	36.3 ± 3.6	91.9 ± 5.7	17.49 ± 2.69	>40
13	CH ₃ 2'-Bromo-phenyl	5.8 ± 2.3	49.8 ± 13.5	18.9 ± 5.5	89.3 ± 6.2	30.19 ± 7.24	>40
14	CH ₃ 2'-Chloro-phenyl	4.3 ± 2.5	12.6 ± 2.7	11.9 ± 0.5	>100	29.92 ± 0.44	>40
15	CH ₃ 2'-Fluoro-phenyl	6.8 ± 0.3	11.9 ± 1.9	34.3 ± 5.9	>100	10.75 ± 3.21	>40
16	CH ₃ 2'-Methoxy-phenyl	36.6 ± 13.1	54.7 ± 9.0	>100	>100	22.73 ± 1.84	7.23 ± 0.97
17	CH ₃ 2'-Methyl-phenyl	19.3 ± 6.7	>100	>100	>100	23.55 ± 3.98	>40
18	OCH ₃ Phenyl	47.3 ± 9.4	33.8 ± 4.9	48.2 ± 7.5	>100	>40	>40
19	OCH ₃ Methyl	>100	>100	>100	>100	>40	>40
20	OCH ₃ Ethyl	>100	>100	>100	>100	>40	>40
21	OCH ₃ Butyl	>100	>100	>100	>100	>40	>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 IC₅₀ values are presented as mean ± SE (*n* = 3).

^c Aspirin, a cyclooxygenase inhibitor, was used as a positive control in platelet aggregation assay.

^d Diphenyleneiodonium (DPI), a NADPH oxidase inhibitor, was used as positive control.

2. Chemistry

2,8-Disubstituted benzoxazinone derivatives, compounds **1–21**,^{21,24–27} (Table 1), were prepared by reaction of 2-amino-3-methoxy-benzoic acid, 2-amino-3-chloro-benzoic acid, and 2-amino-3-methyl-benzoic acid, with corresponding substituted benzoyl chlorides. All products were fully characterized using spectral data.

2.1. Pharmacological evaluation and discussion

As mentioned above, benzoxazinones showed significant inhibition of thrombin and tissue factor VIIa.^{20,28} Both of them were induced pathway of blood coagulation. Furthermore, on the basis of our unpublished data, benzoxazinones also showed prolong thrombin-induced fibrinogen clotting time. Therefore, we focused on the anti-platelet aggregation activities of compounds. Four inducers, AA (arachidonic acid), Col (collagen), PAF (platelet activating factor), and Thr (thrombin), were used in the platelet aggregation assays, and aspirin was also assayed as a positive control.²⁹ The results of the screening are presented in Table 1. Almost all 2-(2'-substituted-phenyl)-benzoxazinones showed inhibitory

effect on Thr, AA, and Col-induced platelet aggregation. Furthermore, except for **8–13**, all compounds were inactive toward PAF-induced aggregation. To our knowledge, benzoxazinones were as thrombin inhibitors, while this is the first time found they also inhibited platelet aggregation which was induced by AA and Col. On the other hand, superoxide anion is considered as an important role in injury to some organs, in which ischemia or inflammation happen. Therefore, finding a modulator of the generation of O₂^{•-} to prevent human diseases is necessary. O₂^{•-} in neutrophils can be induced during phagocytosis and by treatment of the cells with various chemoattractants and activators of protein kinase. However, neutrophil function can be primed by a variety of pro-inflammatory stimuli, and the resting neutrophils in the blood circulation are poorly responsive to agonists. Therefore, the superoxide anion generation of human neutrophils by fMLP (formyl-Met-Leu-Phe) and PMA (phorbol myristate acetate) and the neutrophil elastase release induced by fMLP were examined.^{30–33} Similar to anti-platelet test, almost all 2-(2'-substituted-phenyl)-benzoxazinones showed inhibitory effect on superoxide anion generation and neutrophil elastase release of human neutrophils by fMLP (Table 1), but not the PMA-activated ones. According

to these results, several structure–activity relationship conclusions were summarized from the screen tests:

1. In the anti-platelet aggregation assays of 2-(2'-substituted-phenyl)-benzoxazinones, they showed inhibition on AA-induced effect except for **6**, on collagen-induced effect except for **6** and **17**, and on thrombin-induced effect except for **6**, **16**, and **17**. Compound **6** is unique and displayed strong anti-inflammatory effects related to superoxide anion generation and NE release, whereas vanished in anti-platelet aggregation.
2. The 8-chloro series were exhibited significant inhibitory activities on neutrophils elastase release, but the activities were nil or decrease while the C-8 were replaced by the methyl group. Except for **10**, 8-chloro series were more potent than 8-methoxy and 8-methyl series on superoxide generation of neutrophils. Additionally, 8-chlorine substituted analogues, such as **8**, **9**, and **11**, provide a chance to develop lead drugs with wide-spectrum targets including PAF in both anti-platelet aggregation and anti-inflammatory activities.
3. Compound **14** showed the best inhibition in AA-induced platelet aggregation, as well as significant inhibition in thrombin- and collagen-induced effects, respectively. In the improvement of the activity induced by collagen, the best analogues, compounds **14** and **15**, implied that 8-methyl- and 2'-small halogen substitutions are beneficial.
4. The 2'-methoxyphenyl-benzoxazinones, compounds **6**, **11**, and **16**, are potent on neutrophils elastase release. However, these methoxy-derivatives were inactive or significantly decrease the activities on anti-platelet aggregation induced by AA, collagen, and thrombin.
5. The bioactivities were totally vanished while the presence of 2-(4'-chloro- or 4'-bromo-phenyl)- or 2-alkyl-substitutions.

In conclusion, according to the results of screen tests, the bioactivities of compounds were present a dramatic relationship with the substitution on C-8. This is the first report for the structure–activities relationship studies on C-8 of benzoxazinones. Furthermore, compounds **3**, **4**, **5**, and **7–18** showed significant inhibition on platelet aggregation. Except for **16**, all of them were more potent than aspirin on AA-induced platelet aggregation. These results suggested that benzoxazinones also can be designed and possess aspirin-like activity in addition to their anti-thrombin effects. Additionally, compounds **6** and **16** showed inhibitory effect on neutrophil elastase release and superoxide generation. To our knowledge, benzoxazinones were as neutrophil elastase inhibitors. These results suggested the inhibitory effect on neutrophil elastase release of **6** and **16** may not only by inhibited neutrophile elastase, but also inhibited the neutrophil activation.

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References and notes

1. Malech, H. L.; Gallin, J. I. *N. Eng. J. Med.* **1987**, *317*, 687–694.
2. Witko-Sarsat, V.; Rieu, P.; Descamps-Latscha, B.; Lesavre, P.; Halbwachs-Mecarelli, L. *Lab. Invest.* **2000**, *80*, 617–653.
3. Okajima, K.; Harada, N.; Uchiba, M. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1157–1165.
4. Ennis, M. *Curr. Allergy Asthma Rep.* **2003**, *3*, 159–165.
5. Vinten-Johansen, J. *Cardiovasc. Res.* **2004**, *61*, 481–497.
6. Borregaard, N. *Eur. J. Haematol.* **1998**, *41*, 401–413.
7. Roos, D.; van Bruggen, R.; Meischl, C. *Microbes. Infect.* **2003**, *5*, 1307–1315.
8. Johansson, S.; Goransson, U.; Luijendijk, T.; Backlund, A.; Claesson, P.; Bohlin, L. *J. Nat. Prod.* **2002**, *65*, 32–41.
9. Ross, R. *Nature* **1993**, *362*, 801–809.
10. Schwartz, S. M.; Heimark, R. L.; Majesky, M. W. *Physiol. Rev.* **1990**, *70*, 1177–1209.
11. Wu, C. C.; Wang, W. Y.; Kuo, R. Y.; Chang, F. R.; Wu, Y. C. *Eur. J. Pharmacol.* **2004**, *483*, 187–194.
12. Kurosaki, F.; Naishi, A. *Phytochemistry* **1983**, *22*, 669–672.
13. Ponchet, M.; Favre-Bonvin, J.; Hauteville, M.; Ricci, P. *Phytochemistry* **1988**, *27*, 725–730.
14. Hedsrom, L.; Moorman, A. R.; Dobbs, J.; Abeles, R. H. *Biochemistry* **1984**, *23*, 1753–1759.
15. Krantz, A.; Spencer, R. W.; Tam, T. F.; Liak, T. J.; Copp, L. J.; Thomas, E. M.; Rafferty, S. P. *J. Med. Chem.* **1990**, *33*, 464–479.
16. Bode, W.; Meyer, E., Jr.; Powers, J. C. *Biochemistry* **1989**, *28*, 1951–1963.
17. Neumann, U.; Schechter, N. M.; Gütschow, M. *Bioorg. Med. Chem.* **2001**, *9*, 947–954.
18. Hays, S. J.; Caprathe, B. W.; Gilmore, J. L.; Amin, N.; Emmerling, M. R.; Michael, W.; Nadimpalli, R.; Nath, R.; Raser, K. J.; Stafford, D.; Watson, D.; Wang, K.; Jaen, J. C. *J. Med. Chem.* **1998**, *41*, 1060–1067.
19. Gilmore, J. L.; Hays, S. J.; Caprathe, B. W.; Lee, C.; Emmerling, M. R.; Michael, W.; Jaen, J. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 679–682.
20. Jakobsen, P.; Pedersen, B. R.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2095–2103.
21. Hsieh, P. W.; Chang, F. R.; Chang, C. H.; Cheng, P. W.; Chiang, L. C.; Zeng, F. L.; Lin, K. H.; Wu, Y. C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4751–4754.
22. Chang, F. R.; Wu, C. C.; Hwang, T. L.; Patnam, R.; Kuo, R. Y.; Wang, W. Y.; Lan, Y. H.; Wu, Y. C. *Arch. Pharm. Res.* **2003**, *26*, 511–515.
23. Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639–4750.
24. General experimental procedure for the synthesis of compounds **6** and **7**. To a pyridine solution of 2-amino-3-methoxy-benzoic acid (1.0 mmol) was added with corresponding substituted benzoyl chlorides. The reaction mixture was stirred overnight at room temperature for 16 h. The solvent was evaporated at reduced pressure. The residue was purified by column chromatography (Si-Gel) using CHCl₃/hexane (1:3) mixture to afford the products. The ¹H spectrum was recorded at 400 MHz using CDCl₃ as solvent. Compound **6**: ¹H NMR (CDCl₃): δ 7.87 (1H, dd, *J* = 8.0, 2.0 Hz, Ar-H), 7.79 (1H, dd, *J* = 8.0, 2.0 Hz, Ar-H), 7.50 (1H, td, *J* = 8.0, 2.0 Hz, Ar-H), 7.45 (1H, t, *J* = 8.0 Hz, Ar-H), 7.27 (1H, dd, *J* = 8.0, 1.6 Hz, Ar-H), 7.07 (1H, dd, *J* = 8.0, 1.6 Hz, Ar-H), 6.98 (1H, br d, *J* = 8.0 Hz, Ar-H), 3.98 (3H, s, OMe), 3.90 (3H, s, OMe); EI-MS *m/z*: 283 [M]⁺ (31). Compound **7**: ¹H NMR (CDCl₃): δ 7.98 (1H, dd, *J* = 8.0, 1.6 Hz, Ar-H), 7.78 (1H, dd, *J* = 8.0, 1.6 Hz, Ar-H), 7.42 (1H, t,

- $J = 8.0$ Hz, Ar-H), 7.50 (1H, td, $J = 8.0$, 1.6 Hz, Ar-H), 7.27 (3H, m, Ar-H), 3.97 (3H, s, OMe), 2.70 (3H, s, Me); EI-MS m/z : 267 $[M]^+$ (94).
25. General experimental procedure for the synthesis of compounds **8–12**. To a pyridine solution of 2-amino-3-chloro-benzoic acid (1.0 mmol) was added with corresponding substituted benzoyl chlorides. The reaction mixture was stirred overnight at room temperature for 16 h. The solvent was evaporated at reduced pressure. The residue was purified by column chromatography (Si-Gel) using CHCl_3 /hexane (1:3) mixture to afford the products. The ^1H spectrum was recorded at 400 MHz using CDCl_3 as solvent. Compound **8**: ^1H NMR (CDCl_3): δ 8.18 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.97 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.91 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.75 (1H, dd, $J = 8.0$, 1.2 Hz, Ar-H), 7.50 (1H, t, $J = 8.0$ Hz, Ar-H), 7.45 (1H, dd, $J = 8.0$, 1.2 Hz, Ar-H), 7.39 (1H, td, $J = 8.0$, 1.6 Hz, Ar-H); EI-MS m/z : 337 $[M]^+$ (98), 335 (78). Compound **9**: ^1H NMR (CDCl_3): δ 8.17 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 8.01 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.91 (1H, dd, $J = 8.0$, 1.2 Hz, Ar-H), 7.54 (1H, dd, $J = 8.0$, 1.2 Hz, Ar-H), 7.49 (1H, t, $J = 8.0$ Hz, Ar-H), 7.47 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.41 (1H, td, $J = 8.0$, 1.2 Hz, Ar-H); EI-MS m/z : 291 $[M]^+$ (89), 293 (56). Compound **10**: ^1H NMR (CDCl_3): δ 8.19 (1H, t, $J = 8.0$, 0.8 Hz, Ar-H), 8.15 (1H, d, $J = 8.0$ Hz, Ar-H), 7.89 (1H, d, $J = 8.0$ Hz, Ar-H), 7.56 (1H, m, Ar-H), 7.46 (1H, t, $J = 8.0$ Hz, Ar-H), 7.29 (1H, t, $J = 8.0$ Hz, Ar-H), 7.22 (1H, dd, $J = 8.0$, 1.2 Hz, Ar-H); EI-MS m/z : 275 $[M]^+$ (91), 277 (32). Compound **11**: ^1H NMR (CDCl_3): δ 8.15 (1H, dd, $J = 8.0$, 1.0 Hz, Ar-H), 7.98 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.86 (1H, dd, $J = 8.0$, 1.0 Hz, Ar-H), 7.51 (1H, td, $J = 8.0$, 1.2 Hz, Ar-H), 7.43 (1H, t, $J = 8.0$ Hz, Ar-H), 7.07 (1H, t, $J = 8.0$ Hz, Ar-H), 7.03 (1H, d, $J = 8.0$ Hz, Ar-H), 3.95 (3H, s, OMe); EI-MS m/z : 287 $[M]^+$ (29), 289 (14). Compound **12**: ^1H NMR (CDCl_3): δ 8.13 (2H, dd, $J = 8.0$, 1.2 Hz, Ar-H), 7.86 (1H, dd, $J = 8.0$, 0.8 Hz, Ar-H), 7.42 (1H, m, Ar-H), 7.42 (1H, t, $J = 8.0$ Hz, Ar-H), 7.32 (2H, m, Ar-H), 2.82 (3H, s, Me); EI-MS m/z : 271 $[M]^+$ (100), 273 (33).
26. General experimental procedure for the synthesis of compounds **13–17**. To a pyridine solution of 2-amino-3-methyl-benzoic acid (1.0 mmol) was added with corresponding substituted benzoyl chlorides. The reaction mixture was stirred overnight at room temperature for 16 h. The solvent was evaporated at reduced pressure. The residue was purified by column chromatography (Si-Gel) using CHCl_3 /hexane (1:3) mixture to afford the products. The ^1H spectrum was recorded at 400 MHz using CDCl_3 as solvent. Compound **13**: ^1H NMR (CDCl_3): δ 8.10 (1H, br d, $J = 8.0$ Hz, Ar-H), 7.96 (1H, dd, $J = 8.0$, 2.2 Hz, Ar-H), 7.75 (1H, dd, $J = 8.0$, 1.2 Hz, Ar-H), 7.69 (1H, d, $J = 8.0$ Hz, Ar-H), 7.45 (1H, td, $J = 8.0$, 1.2 Hz, Ar-H), 7.44 (1H, t, $J = 8.0$ Hz, Ar-H), 7.36 (1H, td, $J = 8.0$, 1.6 Hz, Ar-H), 2.64 (3H, s, Me); EI-MS m/z : 315 (100), 317 $[M]^+$ (95). Compound **14**: ^1H NMR (CDCl_3): δ 8.09 (1H, br d, $J = 8.0$ Hz, Ar-H), 8.09 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.69 (1H, dq, $J = 8.0$, 0.8 Hz, Ar-H), 7.56 (1H, dd, $J = 8.0$, 1.2 Hz, Ar-H), 7.45 (1H, td, $J = 8.0$, 1.6 Hz, Ar-H), 7.36 (1H, t, $J = 8.0$ Hz, Ar-H), 7.34 (1H, td, $J = 8.0$, 1.6 Hz, Ar-H), 2.63 (3H, s, Me); EI-MS m/z : 271 $[M]^+$ (100), 273 (35). Compound **15**: ^1H NMR (CDCl_3): δ 8.12 (1H, td, $J = 8.0$, 1.6 Hz, Ar-H), 8.03 (1H, dt, $J = 8.0$, 0.8 Hz, Ar-H), 7.69 (1H, dq, $J = 8.0$, 0.8 Hz, Ar-H), 7.50 (1H, m, Ar-H), 7.37 (1H, t, $J = 8.0$ Hz, Ar-H), 7.25 (1H, td, $J = 8.0$, 1.2 Hz, Ar-H), 7.18 (1H, ddd, $J = 8.0$, 8.0, 1.2 Hz, Ar-H), 2.59 (3H, s, Me); EI-MS m/z : 255 $[M]^+$ (100). Compound **16**: ^1H NMR (CDCl_3): δ 8.08 (1H, dd, $J = 8.0$, 0.8 Hz, Ar-H), 7.92 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 7.65 (1H, dq, $J = 8.0$, 0.8 Hz, Ar-H), 7.49 (1H, ddd, $J = 8.0$, 8.0, 1.6 Hz, Ar-H), 7.38 (1H, t, $J = 8.0$ Hz, Ar-H), 7.06 (1H, td, $J = 8.0$, 0.8 Hz, Ar-H), 7.03 (1H, br d, $J = 8.0$ Hz, Ar-H), 3.93 (3H, s, OMe), 2.61 (3H, s, Me); EI-MS m/z : 267 $[M]^+$ (29). Compound **17**: ^1H NMR (CDCl_3): δ 8.12 (1H, dd, $J = 8.0$, 1.6 Hz, Ar-H), 8.08 (1H, dq, $J = 8.0$, 0.8 Hz, Ar-H), 7.66 (1H, dq, $J = 8.0$, 0.8 Hz, Ar-H), 7.42 (1H, td, $J = 8.0$, 1.2 Hz, Ar-H), 7.40 (1H, t, $J = 8.0$ Hz, Ar-H), 7.33 (2H, m, Ar-H), 2.79 (3H, s, Me), 2.61 (3H, s, Me); EI-MS m/z : 251 $[M]^+$ (100).
27. General experimental procedure for the synthesis of compounds **19–21**. To a pyridine solution of 2-amino-3-methoxy-benzoic acid (1.0 mmol) was added with corresponding acid anhydrides. The reaction mixture was stirred overnight at room temperature for 16 h. The solvent was evaporated at reduced pressure. The residue was purified by column chromatography (Si-Gel) using CHCl_3 /hexane (1:2–1:1) mixture to afford the products. The ^1H spectrum was recorded at 200 MHz using CD_3OD as solvent. Compound **19**: ^1H NMR: δ 7.43 (1H, dd, $J = 2.0$, 7.6 Hz, Ar-H), 7.30 (1H, br t, $J = 8.4$ Hz, Ar-H), 7.22 (1H, dd, $J = 2.0$, 8.4 Hz, Ar-H), 3.87 (3H, s, OMe), 2.11 (3H, s, CH_3); EI-MS m/z : 191 $[M]^+$ (54). Compound **20**: ^1H NMR: δ 7.43 (1H, dd, $J = 8.0$, 1.8 Hz, Ar-H), 7.29 (1H, t, $J = 8.0$ Hz, Ar-H), 7.21 (1H, dd, $J = 8.0$, 1.8 Hz, Ar-H), 3.86 (3H, s, OMe), 2.40 (2H, q, $J = 7.8$ Hz, CH_2CH_3), 1.20 (3H, t, $J = 7.8$ Hz, CH_2CH_3); EI-MS m/z : 205 $[M]^+$ (51). Compound **21**: ^1H NMR: δ 7.43 (1H, dd, $J = 8.0$, 2.0 Hz, Ar-H), 7.29 (1H, t, $J = 8.0$ Hz, Ar-H), 7.22 (1H, dd, $J = 8.0$, 1.8 Hz, Ar-H), 3.87 (3H, s, OMe), 2.39 (2H, t, $J = 7.8$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.67 (2H, m, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.43 (2H, qd, $J = 7.4$, 1.0 Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.20 (3H, t, $J = 7.4$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); EI-MS m/z : 232 $[M-H]^+$ (6).
28. Jakobsen, P.; Horneman, A. M.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2803–2812.
29. Anti-platelet aggregation assays: see Wu, C. C.; Wang, W. Y.; Kuo, R. Y.; Chang, F. R.; Wu, Y. C. *Eur. J. Pharmacol.* **2004**, *483*, 187–194.
30. Neutrophil superoxide anion formation: see Hwang, T. L.; Hung, H. S.; Kao, S. H.; Teng, C. M.; Wu, C. C.; Cheng, S. J. S. *Mol. Pharmacol.* **2003**, *64*, 1419–1427.
31. Measurement of elastase release. Degranulation of azurophilic granules was determined by elastase release as described previously with some modifications.^{32,33} Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils ($5 \times 10^5/\text{ml}$) were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated by fMLP (100 nM)/CB (0.5 $\mu\text{g}/\text{ml}$), and changes in absorbance at 405 nm were continuously monitored to assay elastase release. The results were expressed as the percent of the initial rate of elastase release in the fMLP/CB-activated, drug-free control system.
32. Sklar, L. A.; McNeil, V. M.; Jesaitis, A. J.; Painter, R. G.; Cochrane, C. G. *J. Biol. Chem.* **1982**, *257*, 5471–5475.
33. Coles, B.; Bloodsworth, A.; Clark, S. R.; Lewis, M. J.; Cross, A. R.; Freeman, B. A.; O'Donnell, V. B. *Circ. Res.* **2002**, *91*, 375–381.