Novel Thiazole Derivatives as Inhibitors of Superoxide Production by Human Neutrophils: Synthesis and Structure–Activity Relationships

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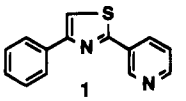
Neutrophils have an important role in the self-defense systems of organisms through the production of superoxide. On the other hand, it has been proposed that abnormal amounts of superoxide produced by neutrophils are a serious factor in tissue injury. A series of novel thiazole derivatives was prepared and evaluated inhibitory effect on superoxide production by human neutrophils *in vitro*. Among these compounds, 6-[2-(3,4-diethoxyphenyl)thiazol-4-yl]-pyridine-2-carboxylic acid (OPC-6535) was selected as one of the most promising compounds. The synthesis and structure–activity relationships of these compounds are reported herein.

It has long been believed that neutrophils play a main role in the protection of the host from infections, since activated neutrophils attack infectious bacteria through the release of active oxygen species such as hydrogen peroxide, hydroxy radical, and hypochlorite which are converted from the superoxide produced by neutrophils. Actually, it has been reported that acquired and congenital severe abnormalities of neutrophil functions have led patients into recurrent bouts of pneumonia and other types of life-threatening bacterial and fungal infections.[1] However, it has become apparent that neutrophils have not only useful effects but also deleterious ones on organ systems. Recent studies have shown that normal host tissues in abnormal environments might be inappropriately recognized as targets by neutrophils[2] due either to the low intrinsic ability of neutrophils to differentiate between foreign and host antigens or to the susceptibility of neutrophils to be activated by intrinsic immune factors, such as antibodies, complements, and cytokines. Although normal tissues have many defense and healing systems against such oxygen injuries, abnormal environments such as ischemia, trauma, and the activation of the autoimmune system blunt these defensive systems. Furthermore, the uncontrolled and excessive production of superoxide by neutrophils will affect these tissues, and consequently, undesirable tissue injuries will be caused by these active oxygen species. In other words, neutrophils are one of the major mediators of tissue injuries in inflammatory diseases, including myocardial ischemia–reperfusion injury, coronary artery diseases,[3] rheumatoid arthritis, adult respiratory distress syndromes, multiple organ failure, blistering skin disorders, and ulcerative colitis. This understanding has encouraged us to develop therapeutic interventions that attenuate the injuries induced by neutrophils.

To prevent injury from superoxide, two approaches seem to be effective. One is to scavenge the superoxide, and the other is to inhibit the production of superoxide. Various types of superoxide scavengers have been known, such as superoxide dismutase (SOD),[4] SOD mimic,[5] and others.[6] Among these scavengers, SOD has been expected to cure the myocardial ischemia–reperfusion injury[7] and inflammatory disease[8] since its discovery in 1969.[9] Furthermore, many types of SOD[10] have been developed to improve the bioavailability and distribution to the focus. On the other hand, while a large number of compounds[11] including anti-inflammatory, antirheumatic drugs, natural products, and antibiotics with a *slight inhibition* other than their main efficacy have been estimated, to our knowledge, there have been no drug developed as an inhibitor of superoxide production. Therefore, we made our strategy to search for a new type of drug which would act as an inhibitor rather than a scavenger of superoxide. We performed random screening tests of various compounds for inhibitory activity against superoxide production. These tests identified the simple compound **1** ($IC_{50} = 1 \times 10^{-6}$ M). Thus we report herein on the further modifications of lead compound **1** and on the structure-activity relationships.



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Chemistry

Schemes 1–3 show a facile synthesis of thiazole, oxazole, and imidazole derivatives. The thiazole skeleton was prepared by Hantzsch's method,[12] which is a condensation of α-halo ketone **7** or **17** and thioamide **6**. α-Halo ketones were obtained by Friedel–Crafts reaction of 3,4-dihydro-2(1*H*)-quinolinone[13] or bromination of acetyl compounds **16**. Thioamides were synthesized from various nitriles **5** by Taylor's method.[14] Corresponding α-halo ketones and thioamides were refluxed in alcohol for 1-6 h to afford thiazoles **8** and **18** in good yields. Further conversion of substituents gave **9** and **19**. Introduction of a carbonyl or hydroxymethyl moiety (**8d,e**) was obtained by CrO_3 oxidation of methylene compound **8c**, followed by $NaBH_4$ reduction. Oxazole compound **12** was obtained by cyclization of α-(acyloxy) ketone **11** with NH_4OAc, which was prepared from a sodium salt of 3,4-dimethoxybenzoic acid (**10**) and 6-(chloroacetyl)-3,4-dihydro-2(1*H*)-quinolinone (**7**). Imidazole compound **15** was synthesized by the same procedure as oxazole. Gabriel reaction from **7** gave amine **13**, and acylation of **13** with 3,4-dimethoxybenzoyl chloride provided amide **14**. Cyclization of **14** with

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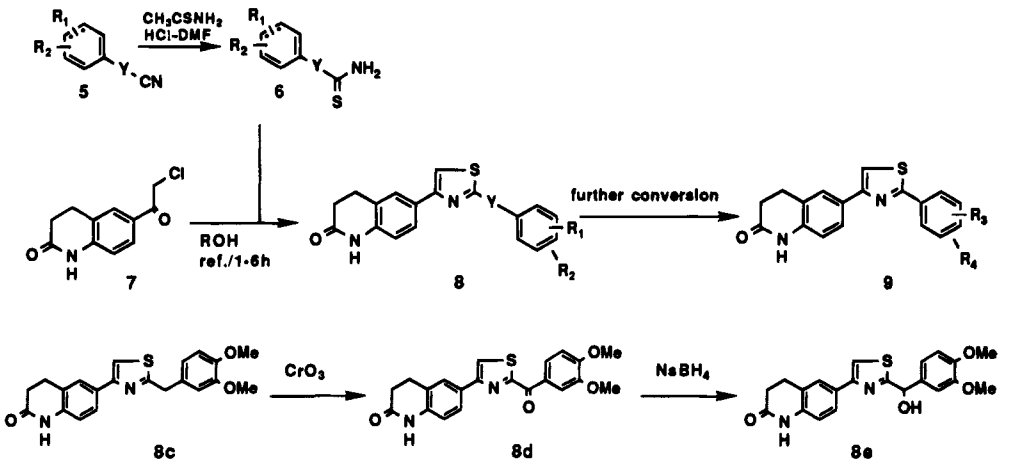
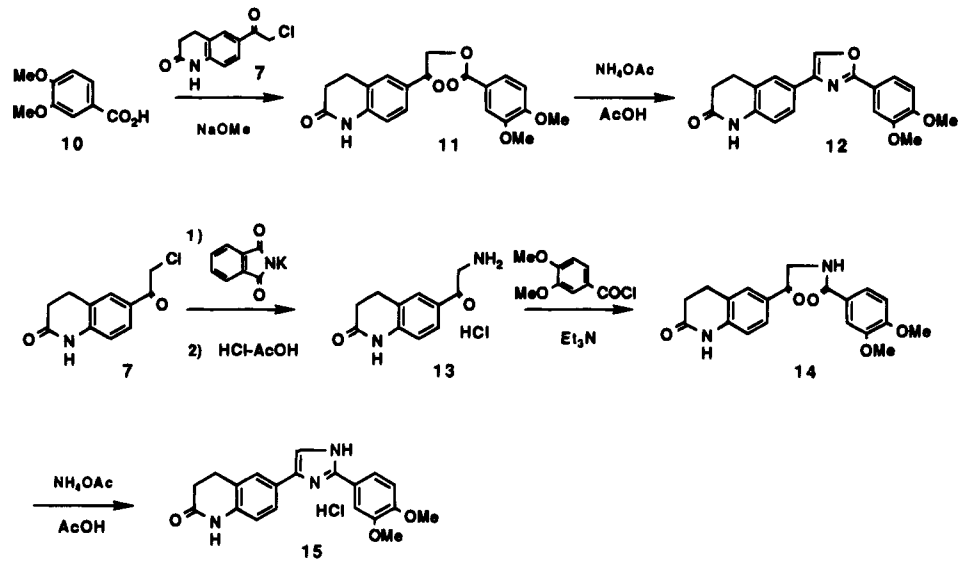
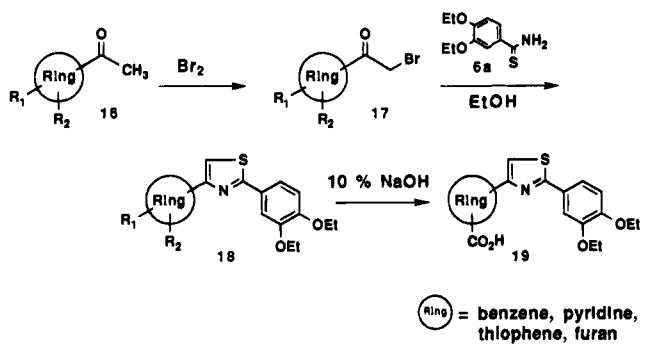
Scheme 1



Scheme 2



Scheme 3



NH_4OAc afforded imidazole **15**. The synthesized thiazole, oxazole, and imidazole compounds are shown in Tables 1–4.

Results and Discussion

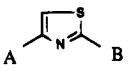
The synthesized compounds were evaluated for their inhibitory activities against superoxide production by human neutrophils in vitro. Human neutrophils were isolated from fresh venous blood of healthy adults using standard dextran sedimentation and gradient separation by the Ficoll-paque method.[15] Superoxide was determined by the SOD inhibitable cytochrome c reduction method using formyl methionyl peptide (f-MLP) as a stimulant.[16]

Firstly, identified compound **1** ($IC_{50} = 1 \times 10^{-6}$ M) was roughly modified to various thiazole derivatives. Rings at thiazole C-2 and C-4 were converted to a substituted phenyl or N-containing ring, such as aniline, pyridine, and 3,4-dihydro-2(1*H*)-quinolinone (carbostyril), which was a familiar skeleton in our laboratory.[17] While the activities of other derivatives were at same level as that of **1**, compound **8b** only showed enhanced activity ($IC_{50} = 3 \times 10^{-7}$ M), as shown in Table 1. So we selected **8b** and started to reveal the structure–activity relationships in details.

As the simple compound **8b** was comprised of three aromatic rings, we tried to change the moiety of each to obtain candidate compounds in steps as follow: (1) the thiazole ring of **8b** was replaced with other heteroaromatic rings, (2) the most appropriate substituent on the right phenyl ring was determined, (3) the left carbostyril ring was converted to optimize the activity, and (4), finally, solubility and safety were pursued for development of the drug.

The replacement of the thiazole ring with other heteroaromatic rings (**12**, **15**) did not improve activity, as shown in Table 2. Thus, a thiazole ring was the optimum central group. The center and left parts of the screening compounds were fixed with thiazole and carbostyril. Methylene, carbonyl, hydroxymethyl, and vinyl moieties (**8c-f**) were inserted between the thiazol and 3,4-dimethoxyphenyl rings. All these compounds

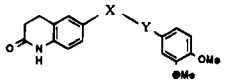
Table 1. Physical Properties and in Vitro Activities Associated with Substituted Rings at Thiazole C-2 and C-4



compound	A	B	mp, °C[a]	formula[b]	IC_{50}, μM[c]
1	ph	py	67–68	$C_{14}H_{10}N_2S$	1
2	4-$NH_2C_6H_4$	py	172–173	$C_{14}H_{11}N_3S$[e]	3
3	py	py	128–129	$C_{13}H_9N_3S$	1
4	6-carbostyril[d]	py	200–202	$C_{17}H_{13}N_3OS{\cdot}HCl$[f]	1
8a	6-carbostyril	ph	221–222	$C_{18}H_{14}N_2OS$	>10
8b	6-carbostyril	3,4-$(MeO)_2C_6H_3$	205–206	$C_{20}H_{18}N_2O_3S{\cdot}{}^1/_4H_2O$	0.3

[a] All compounds were purified by recrystallization from the appropriate solvent. [b] Analyses within ±0.4% for C, H, and N were obtained for all indicated formulas, unless otherwise stated. [c] Inhibitory activity against superoxide production from human neutrophils stimulated with f-MLP. [d] Carbostyril = 3,4-dihydro-2(1*H*)-quinolinone. [e] C: calcd, 66.38; found, 65.91. [f] C: calcd, 59.38; found, 58.90.

Table 2. Physical Properties and in Vitro Activities Associated with Heteroaromatic Rings and Insertion between the Thiazole and Phenyl Rings



compound	X	Y	mp,°C[a]	formula[b]	IC_{50}(μM)[c]
8b		—	205-206	$C_{20}H_{18}N_2O_3S{\cdot}1/4H_2O$	0.3
12		—	191-192	$C_{20}H_{18}N_2O_4$	>10
15		—	276-279	$C_{20}H_{19}N_3O_3{\cdot}HCl$	>10
8c		—CH_2—	185-186	$C_{21}H_{20}N_2O_3S{\cdot}HCl$	10
8d		—CO—	249-251	$C_{21}H_{18}N_2O_4S{\cdot}1/2H_2O$	1.0
8e		—CHOH—	188-189	$C_{21}H_{20}N_2O_4S{\cdot}1/3H_2O$[e]	NE[d]
8f			182-183	$C_{22}H_{20}N_2O_3S{\cdot}1/3H_2O$	2.8

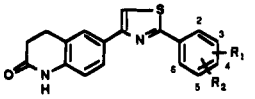
[a] All compounds were purified by recrystallization from the appropriate solvent. [b] Analyses within ±0.4% for C, H, and N were obtained for all indicated formulas, unless otherwise stated. [c] Inhibitory activity against superoxide production from human neutrophils stimulated with f-MLP. [d] NE = no effect. [e] N: calcd, 6.96; found, 6.41.

were less active than **8b**. Analogues of **8b** with single substituents on the right phenyl ring (**8g–j**) afforded no active compound except the compound with the methoxy substituent (**8g**), as shown in Table 3. Evaluation of analogues with two substituents on this ring (Table 3, **8b,k,l, 9a–c**) revealed that electron-withdrawing substituent also decreased the activity. From these results, our attention had been focused on the dialkoxy substituent on the phenyl ring. Among the isomers (**8b,m–o**), the 3,4-positions proved to be more active than the others, in contrast to the inactive 3,5-positions. Surprisingly, the 2,3-positions increased superoxide production. The effect of changes in the alkoxy substituents on the phenyl ring was studied (**8b,p–z, 9d**) and the activity was markedly changed by the carbon chain length of the alcohol, with a one- to three-membered carbon chain being optimum. Enhanced activity was obtained for diethoxy compound **8p**, but not for cyclic ether **8z**. On the basis of these results, we chose 2-(3,4-diethoxyphenyl)thiazole as the center and right part and began modification of the carbostyril functionality (Table 4). Compounds with various substituted-phenyl and heteroaromatic rings at thiazole C-4 were prepared and evaluated (compounds are not shown). A simple 3,5-diacetoxy phenyl derivative (**18a**) showed the strongest activity among the compounds; however, this compound and the previously described carbostyril series were not sufficiently soluble in aqueous solution. Our goal was to develop an injectable agent which could be used for acute diseases, such as myocardial infarction, so it was necessary to search for a soluble compound. Therefore, we next turned our attention to the search for a soluble compound with consideration of the above-mentioned structure–activity relationships. Introduction of a soluble function on the left aromatic ring remarkably decreased the activity though it increased the solubility. After further examination, it was found that a sodium salt of carboxylic acid **19a** could solve this problem, and compound **19b** proved to be most active among the isomers. Compounds **19d–f**, which were desirable in both solubility and activity, were elaborated with the consideration of carboxylic acid's position. We finally selected **19f** as a promising compound with not only satisfactory activity and solubility but also safety, which are indispensable for a drug.

We also evaluated all of the compounds for superoxide production stimulated by phorbol myristate acetate (PMA) to check whether or not these compounds are simple scavengers. A scavenger will show *equal activity* against different stimulants. These compounds, active against f-MLP-stimulated neutrophils, did not show inhibition of PMA-stimulated superoxide production. This result suggested that the synthesized compounds were not superoxide scavengers. We confirmed directly that compound **19f** is not a scavenger by measurement with electron spin resonance (ESR), in which compound **19f** did not scavenge the superoxide produced from a hypoxanthine-xanthine oxidase system.[18] The inhibition mechanism of superoxide production is not yet clear, but from the evidence of inhibition pattern by two stimulants (f-MLP or PMA), it is improbable that compound **19f** inhibits superoxide production by acting on NADPH-oxidase or protein kinase C (PKC) directly. (If compound **19f** acts on NADPH-oxidase or PKC, PMA-stimulated superoxide production is also inhibited.)

In conclusion, we were able to elaborate novel thiazole compounds that were simple non-peptide molecules different from SOD as extremely strong inhibitors of superoxide production by human neutrophils. The structure–activity relationships were studied, and 2-(3,4-diethoxyphenyl)-4-(3,5-diacetoxyphenyl)thiazole (**18a**) exhibited the strongest activity (IC_{50} = 0.001 μM) among these compounds. Compound **19f**, with a picolinic acid moiety, is a soluble compound and will be capable of being administered *in vivo*. Although many scavengers have been previously developed, our compound has a unique profile which acts on the human neutrophils themselves to inhibit superoxide production. It is not yet clear if our approach is desirable for actual disease

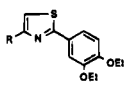
Table 3. Physical Properties and in Vitro Activities Associated with Substituents on the Phenyl Ring



compd	R_1	R_2	mp, °C[a]	formula[b]	IC_{50}, μM[c]
8g	H	4-OMe	216–217	$C_{19}H_{16}N_2O_2S$	9
8h	H	4-Cl	263–264	$C_{18}H_{13}ClN_2OS$	NE[d]
8i	H	4-NO_2	>300	$C_{18}H_{13}N_3O_3S$	NE
8j	H	4-CO_2H	>300	$C_{19}H_{14}N_2O_3S$	NE
8k	3-Me	4-Me	231–232	$C_{20}H_{18}N_2OS$	NE
8b	3-OMe	4-OMe	205–206	$C_{20}H_{18}N_2O_3S \cdot {}^1/_4H_2O$	0.3
8l	3-OMe	4-SMe	255–256	$C_{20}H_{18}N_2O_2S_2$	0.4
9a	3-OMe	4-SOMe	264–265	$C_{20}H_{18}N_2O_3S_2$	0.8
9b	3-OMe	4-SO_2Me	284–286	$C_{20}H_{18}N_2O_4S_2$	NE
9c	3-OAc	4-OAc	209–210	$C_{22}H_{18}N_2O_5S$	NE
8m	2-OMe	3-OMe	235–236	$C_{20}H_{18}N_2O_3S$	act.[e]
8n	2-OMe	4-OMe	236–237	$C_{20}H_{18}N_2O_3S \cdot HCl$[f]	3
8o	3-OMe	5-OMe	184–185	$C_{20}H_{18}N_2O_3S \cdot {}^1/_4H_2O$	NE
9d	3-OH	4-OH	255–258	$C_{18}H_{14}N_2O_3S \cdot HBr$	1
8p	3-OEt	4-OEt	191–192	$C_{22}H_{22}N_2O_3S \cdot {}^1/_4H_2O$[g]	0.08
8q	3-OC_3H_7	4-OC_3H_7	203–204	$C_{24}H_{26}N_2O_3S$	NE
8r	3-OC_4H_9	4-OC_4H_9	170–171	$C_{26}H_{30}N_2O_3S$	NE
8s	3-$OC_{10}H_{21}$	4-$OC_{10}H_{21}$	126–127	$C_{38}H_{54}N_2O_3S \cdot {}^1/_4H_2O$	NE
8t	3-OMe	4-OEt	203–204	$C_{21}H_{20}N_2O_3S \cdot {}^1/_4H_2O$[h]	0.6
8u	3-OMe	4-OC_3H_7	164–165	$C_{22}H_{22}N_2O_3S$	NE
8v	3-OMe	4-OC_4H_9	185–186	$C_{23}H_{24}N_2O_3S$	NE
8w	3-OEt	4-OMe	179–181	$C_{21}h_{20}N_2O_3S$	0.09
8x	3-OC_3H_7	4-OMe	188–189	$C_{22}H_{22}N_2O_3S \cdot {}^1/_4H_2O$	0.09
8y	3-OC_4H_9	4-OMe	189–190	$C_{23}H_{24}N_2O_3S$	0.3
8z	3,4-OCH_2CH_2O–		191–192	$C_{20}H_{16}N_2O_3S$	NE

[a] All compounds were purified by recrystallization from the appropriate solvent. [b] Analyses within ±0.4% for C, H, and N were obtained for all indicated formulas, unless otherwise stated. [c] Inhibitory activity against superoxide production from human neutrophils stimulated with f-MLP. [d] NE = no effect. [e] act. = activation of superoxide production. [f] N: calcd, 6.95; found, 7.50. [g] N: calcd, 7.02; found, 6.41. [h] N: calcd, 7.19; found, 6.45.

Table 4. Physical Properties, in Vitro Activities and Solubilities Associated with Substituents at Thiazole C-4



compound	R	mp,°C[a]	formula[b]	IC_{50}(μM)[c]	solubility(mg/ml)[d]
8p		191-192	$C_{22}H_{22}N_2O_3S \cdot 1/4H_2O$	0.08	ND[e]
18a		137-138	$C_{23}H_{23}NO_6S$	0.001	<0.001
19a		219-220	$C_{20}H_{19}NO_4S$	0.2	23[f]
19b		192-193	$C_{20}H_{19}NO_4S$	0.005	23[f]
19c		131-132	$C_{20}H_{19}NO_4S$	>10	21[f]
19d		207-209	$C_{18}H_{17}NO_5S$	0.02	23[f]
19e		190-191	$C_{18}H_{17}NO_4S_2$	0.08	23[f]
19f		182-184	$C_{19}H_{18}N_2O_4S$	0.07	21[f]

[a] All compounds were purified by recrystallization from the appropriate solvent. [b] Analyses within ±0.4% for C, H, and N were obtained for all indicated formulas, unless otherwise stated. [c] Inhibitory activity against superoxide production from human neutrophils stimulated with f-MLP. [d] Solubility was measured by shaking with water, followed by detection with HPLC. [e] ND = not detectable. [f] Solubility was measured by shaking with water and 1 equiv of NaOH, followed by detection with HPLC.

treatment. Therefore, in the future we hope to elucidate this point using our compound in vivo, not only for reperfusion injury but also for other inflammations as well.

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on either a Bruker AC-200 (200 MHz) spectrometer or a Bruker AC-250 (250 MHz) spectrometer with Me_4Si as an internal standard. Melting points were determined on a Yanagimoto Micro Point apparatus and were uncorrected. Elemental analysis was performed on a Yanaco MT-5 CHN CORDER, and results were within ±0.4% of the theoretical values unless noted otherwise. The following generalized experimental procedures were utilized to prepare the thioamides and thiazoles described in this report. All α-halo ketones were used without further purification in order to avoid the decomposition which would result from the purification process.

General Procedure for Thioamide Preparation from Nitrile. 3,4-Diethoxybenzthioamide[19] (6a). A mixture of 3,4-diethoxybenzonitrile (**5a**) (270 g, 1.4 mol) and thioacetamide (211 g, 2.8 mol) in 10% HCl–DMF solution (1.5 l) was stirred at 100 °C for 2 h. After cooling, the reaction mixture was poured onto ice. The precipitate was filtered and washed with Et_2O. The crude product was recrystallized from EtOAc to give **6a** (235 g, 74.5%) as yellow plates: mp 153–154 °C (lit. mp 157–158 °C); ^{1}H NMR ($CDCl_3$) δ 1.33 (6H, t, J = 7 Hz), 4.04 (2H, q, J = 7 Hz), 4.07 (2H, q, J = 7 Hz), 6.95 (1H, d, J = 9.1 Hz), 7.65–7.50 (2H, m), 9.30 (1H, br s), 9.62 (1H, br s).

General Procedure for Condensation of α-Halo Ketone and Thioamide. 6-[2-(3,4-Diethoxyphenyl)thiazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone (8p). To a stirred solution of **6a** (1 g, 4.4 mmol) in EtOH (20 mL) was added 6-(chloroacetyl)-3,4-dihydro-2(1*H*)-quinolinone[13] (**7**) (990 mg, 4.4 mmol) and the mixture refluxed for 2 h. After cooling of the solvent, the precipitate was collected and recrystallized from 1,4-dioxane to afford **8p** (1.1 g, 63.4%) as colorless needles: mp 191–192 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, J = 7 Hz), 1.37 (3H, t, J = 7 Hz), 2.49 (2H, t, J = 6.8 Hz), 2.95 (2 H, t, J = 6.8 Hz), 4.09 (2H, q, J = 7 Hz), 4.13 (2H, q, J = 7 Hz), 6.92 (1H, d, J = 8 Hz), 7.06 (1H, d, J = 8.6 Hz), 7.47–7.58 (2H, m), 7.73–7.89 (2H, m), 7.90 (1H, s), 10.18 (1H, s). Anal. ($C_{22}H_{22}N_2O_3S \cdot {}^1/_4H_2O$) C, H, N.

6-[2-(3,4-Dimethoxybenzoyl)thiazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone (8d). To a solution of **8c** (2 g, 5.3 mmol) in AcOH (50 mL) was added CrO_3 (1.2 g, 5.3 mmol), and

stirring was continued for 3 h at 70 °C. Florisil (2 g) was added to this reaction mixture and the mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was dissolved in $CHCl_3$/MeOH (4:1) and filtered. Evaporation of the solvent gave a crude product, which was purified by column chromatography ($CHCl_3$:MeOH = 199:1), followed by recrystallization from $CHCl_3$/EtOH to afford **8d** (300 mg, 14.4%) as pale brown needles: mp 249–251 °C; ^{1}H NMR ($CDCl_3$) δ 2.69 (1H, d, J = 7 Hz), 2.72 (1H, d, J = 8 Hz), 3.06 (1H , d, J = 8 Hz), 3.09 (1H, d, J = 7 Hz), 4.01 (6H, s), 6.88 (1H, d, J = 8.7 Hz), 7.03 (1H, d, J = 8.6 Hz), 7.72–7.86 (2H, m), 7.76 (1H, s), 8.20 (1H, d, J = 2 Hz), 8.45 (1 H, br s), 8.52 (1H, dd, J = 2.0, 8.6 Hz). Anal. ($C_{21}H_{18}N_2O_4S \cdot {}^1/_2H_2O$) C, H, N.

6-[2-{1-(3,4-Dimethoxyphenyl)-1-hydroxymethyl}-thiazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone (8e). To a stirred solution of **8d** (100 mg, 0.25 mmol) in MeOH/$CHCl_3$ (10 mL; 2:3) was added portionwise $NaBH_4$ (100 mg, 0.25 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature. After evaporation of the solvent, the residue was extracted with $CHCl_3$, washed with H_2O, and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was purified by column chromatography ($CHCl_3$: MeOH = 99:1), followed by recrystallization from EtOAc to afford **8e** (52 mg, 52.5%) as pale brown prisms: mp 188–189 °C; ^{1}H NMR ($CDCl_3$) δ 2.61 (1H, d, J = 7 Hz), 2.65 (1H, d, J = 8 Hz), 2.95 (1H, d, J = 8 Hz), 2.99 (1H, d, J = 7 Hz), 3.86 (6H, s), 4.26 (1H, d, J = 3.3 Hz), 6.05 (1H, d, J = 3.3 Hz), 6.79 (1H, d, J = 8.2 Hz), 6.85 (1H, d, J = 8.7 Hz), 7.00–7.10 (2H, m), 7.33 (1H, s), 7.61 (1H, dd, J = 1.8, 8.2 Hz), 7.67 (1H, br s), 9.10 (1H, br s). Anal. ($C_{21}H_{20}N_2O_4S \cdot {}^1/_2H_2O$) C, H, N.

6-[2-(3-Methoxy-4-(methylsulfinyl)phenyl)thiazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone (9a). To a stirred solution of **81** (3.4 g, 8.9 mmol) in $CHCl_3$/EtOH (150 mL; 1:1) was added portionwise *m*-chloroperoxybenzoic acid (2 g, 9 mmol) at 0 °C. The reaction mixture was stirred for 14 h at room temperature. After evaporation of the solvent, the residue was extracted with $CHCl_3$, washed with saturated aqueous Na_2CO_3, and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was recrystallized from DMF to give **9a** (500 mg, 14%) as pale yellow needles: mp 264–265 °C; ^{1}H NMR (DMSO-d_6) δ 2.40–2.60 (2H, m), 2.76 (3H, s), 2.94 (1H, d, J = 7.8 Hz), 2.98 (1H, d, J = 7 Hz), 4.00 (3H, s), 6.94 (1H, d, J = 8.2 Hz), 7.68 (1H, s), 7.72–7.80 (4H, m), 8.09 (1H, s), 10.20 (1H, s). Anal. ($C_{20}H_{18}N_2O_3S_2$) C, H, N.

6-[2-(3-Methoxy-4-(methylsulfonyl)phenyl)thiazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone (9b). To a stirred solution of **9a** (2.9 g, 7.3 mmol) in $CHCl_3$/EtOH (100 mL; 1:1) was added portionwise *m*-chloroperoxybenzoic acid (1.7 g, 8 mmol) at 0 °C. The reaction mixture was stirred for 14 h at room temperature. The precipitate was filtered and recrystallized from DMF/H_2O to give **9b** (1.2 g, 39%) as a white powder: mp 284–286 °C; ^{1}H NMR (DMSO-d_6) δ 2..40–2.50 (2H, m), 2.90–3.04 (2H, m), 3.29 (3H, s), 4.10 (3H, s), 6.94 (1H, d, J = 8 Hz), 7.70–7.95 (5H, m), 8.16 (1H, s), 10.20 (1H, s). Anal. ($C_{20}H_{18}N_2O_4S_2$) C, H, N.

6-[2-(3,4-Dihydroxyphenyl)thiazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone Hydrobromide (9d). To a stirred solution of **8b** (500 mg, 1.3 mmol) in AcOH (4 ml) was added hydrobromic acid (2 mL). The reaction mixture was stirred at reflux for 8 h. After cooling of the reaction mixture, precipitate was filtered and recrystallized from EtOH to give **9d** (270 mg, 47.3%) as an yellow powder: mp 255–258 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.37–2.55 (2H, m), 2.89 (1H, d, J = 8.2 Hz), 2.93 (1H, d, J = 6.8 Hz), 6.80 (1H, d, J = 8.2 Hz), 6.88 (1H, d, J = 8.2 Hz), 7.24 (1H, dd, J = 2.2, 8.2 Hz), 7.41 (1H, d, J = 2.2 Hz), 7.69–7.85 (1H, m), 7.80 (1H, s), 10.16 (1H, s). Anal. ($C_{18}H_{14}N_2O_3S \cdot HBr$) C, H, N.

6-[2-(3,4-Diacetoxyphenyl)thiazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone (9c). A mixture of **9d** (660 mg, 1.6 mmol), acetic anhydride (10 mL), and pyridine (10 ml) was stirred at room temperature for 2 days. The precipitate was filtered and washed, followed by recrystallization from CH_3CN to provide **9c** (390 mg, 58.8%) as colorless needles: mp 209–210 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 2.32 (3H, s), 2.42–2.55 (2H, m), 2.90–3.06 (2H, m), 6.92 (1H, d, J = 8.1 Hz), 7.43 (1H, d, J = 9 Hz), 7.80–7.95 (4H, m), 8.05 (1H, s), 10.20 (1H, s). Anal. ($C_{22}H_{18}N_2O_5S$) C, H, N.

6-[2-((3,4-Dimethoxybenzoyl)oxy)acetyl]-3,4-dihydro-2(1*H*)-quinolinone (11). A mixture of 3,4-dimethoxybenzoic acid (**10**) (2 g, 11 mmol) and sodium methoxide (600 mg, 11 mmol) in MeOH (80 mL) was stirred for 30 min at room temperature. After evaporation of the solvent, 6-(chloroacetyl)-3,4-dihydro-2(1*H*)-quinolinone (**7**) (990 mg, 4.4 mmol) and DMF (50 mL) were added to the residue, and stirring was continued for 2 h at 140 °C. Much of the solvent was removed in vacuo and the addition of water to the reaction mixture gave a precipitate, which was recrystallized from DMF/H_2O to afford **11** (3.8 g, 93.5%) as a white powder: mp 215–216 °C; ^{1}H NMR (DMSO-d_6) δ 2.53 (2H, t, J = 6 Hz), 3.00 (2H, t, J = 6 Hz), 3.84 (3H, s), 3.88 (3H, s), 5.64 (2H, s), 7.01 (1H, d, J = 8 Hz), 7.13 (1H, d, J = 8.6 Hz), 7.51 (1H, d, J = 2 Hz), 7.68 (1H, dd, J = 2, 8.6 Hz), 7.80–7.92 (2H, m), 10.50 (1H, s). Anal. ($C_{20}H_{19}NO_6$) C, H, N.

6-[2-(3,4-Dimethoxyphenyl)oxazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone (12). A mixture of **11** (500 mg, 1.4 mmol) and NH_4OAc (5 *g*, 65 mmol) in AcOH (25 mL) was refluxed for 16 h. After evaporation of the solvent, the residue was extracted with $CHCl_3$, washed with saturated aqueous NaCl, and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was recrystallized from EtOH to give **12** (300 mg, 63.4%) as white needles: mp 191–192 °C; ^{1}H NMR (DMSO-d_6) δ 2.51 (2H, t, J = 7 Hz), 2.96 (2H, t, J = 7 Hz), 3.86 (3H, s), 3.89 (3H, s), 6.93 (1H, d, J = 8 Hz), 7.14 (1H, d, J = 8.4 Hz), 7.50–7.72 (4H, m), 8.55 (1H, s), 10.20 (1H, s). Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

6-(Aminoacetyl)-3,4-dihydro-2(1*H*)-quinolinone Hydrochloride (13). A mixture of 6-(chloroacetyl)-3,4-dihydro-2(1*H*)-quinolinone (**7**) (10 g, 44.7 mmol) and potassium phthalimide (9.1 g, 49.2 mmol) in DMF (100 mL) was stirred for 16 h at room temperature. The reaction mixture was poured into water, and the precipitate was filtered and dried. The crude product was used in next step without further purification.

To a stirred solution of the above product in AcOH (50 mL) was added concentrated HCl (50 mL), and stirring was continued for 24 h at reflux. After evaporation of the solvent, the residue was dissolved in water, and insoluble solid was filtered off. The addition of acetone to the filtrate gave a precipitate which was recrystallized from acetone/H_2O to afford **13** (6.5 g, 60.4%) as a white powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 2.52 (2H, t, J = 8 Hz), 2.98 (2H, t, J = 7.8 Hz), 4.48 (2H, br s), 6.99 (1 H, d, J = 8.2 Hz), 7.80–7.91 (2H, m), 8.38 (3H, br s), 10.57 (1H, s). Anal. ($C_{11}H_{12}N_2O_2 \cdot HCl$) C, H, N.

6-[(3,4-Dimethoxybenzamido)acetyl]-3,4-dihydro-2(1*H*)-quinolinone (14). To a stirred solution of **13** (3 g, 12.5 mmol) and Et_3N (7 mL, 50 mmol) in THF (60 mL) was added 3,4-dimethoxybenzoyl chloride (2.8 g, 13.7 mmol) at 0 °C. After stirring was continued for 3 h, the precipitate was collected, washed with MeOH, and dried. Recrystallization from MeOH gave **14** (2.6 g, 55.4%) as white needles: mp 246–247 °C; ^{1}H NMR (DMSO-d_6) δ 2.47 (2H, t, J = 8.2 Hz), 2.94 (2H, t, J = 7.9 Hz), 3.77 (3H, s), 3.78 (3H, s), 4.66 (2H, d, J = 5.5 Hz), 6.92 (1H, d, J = 8.1 Hz), 7.00 (1H, d, J = 8.4 Hz), 7.49–7.55 (2H, m), 7..78–7.92 (2H, m), 8.63 (1H, t, J = 5.5 Hz), 10.43 (1H, s). Anal. ($C_{20}H_{20}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

6-[2-(3,4-Dimethoxyphenyl)imidazol-4-yl]-3,4-dihydro-2(1*H*)-quinolinone Hydrochloride (15). A mixture of **14** (500 mg, 1.4 mmol) and NH_4OAc(10 g, 130 mmol) in AcOH (25 mL) was refluxed for 48 h. After evaporation of the solvent, the residue was partitioned between 2% HCl and $CHCl_3$. The aqueous layer was neutralized with 10% NaOH, extracted with EtOAc, washed with saturated aqueous NaCl, and dried over $MgSO_4$. The addition of HCl/EtOH and evaporation of the solvent gave a crude product, which was recrystallized from EtOH to afford **15** (180 mg, 34.6%) as a gray powder. Material **14** (250 mg, 50%) was recovered from the above $CHCl_3$ layer: mp 276–279 °C; ^{1}H NMR (DMSO-d_6) δ 2.48 (2H, t, J = 8 Hz), 2.95 (2H, t, J = 8 Hz), 3.86 (3 H, s), 3.89 (3H, s), 6.96 (1H, d, J = 8.1 Hz), 7.22 (1H, d, J = 8.6 Hz), 7.73–7.87 (3H, m), 7.91 (1H, d, J = 2.1 Hz), 8.07 (1H, s), 10.28 (1H, s). Anal. ($C_{20}H_{19}N_3O_3 \cdot HCl$) C, H, N.

Methyl 6-[2-(3,4-Diethoxyphenyl)thiazol-4-yl]pyridine-2-carboxylate (18b). To a stirred solution of 6-acetylpyridine-2-carboxylic acid[20] (**16a**) (30 g, 179.3 mmol) in AcOH (250 mL) was added portionwise bromine (28.7 g, 179.3 mmol). The reaction mixture was stirred at 110 °C for 10 min. A color change from deep red to colorless was observed. Evaporation of the solvent gave **17a**, which was used immediately without further purification.

A mixture of the above **17a** and **6a** (35 g, 155 mmol) in MeOH (600 mL) was stirred at reflux for 4 h. Half of the solvent was removed *in vacuo* and the precipitate was filtered. Recrystallization from EtOAc provided **18b** (49 g, 71%) as a white powder: mp 123–124 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (3H, t, J = 7 Hz), 1.52 (3H, t, J = 7 Hz), 4.03 (3H, s), 4.17 (2H, q, J = 7 Hz), 4.23 (2H, q, J = 7 Hz), 6.93 (1H, d, J = 8.4 Hz), 7.54 (1H, dd, J = 2.1, 8.4 Hz), 7.64 (1H, d, J = 2.1 Hz), 7.95 (1H, t, J = 7.7 Hz), 8.06 (1H, dd, J = 1.3, 7.7 Hz), 8.26 (1H, s), 8.46 (1H, dd, J = 1.3, 7.7 Hz). Anal. ($C_{20}H_{20}N_2O_4S$) C, H, N.

6-[2-(3,4-Diethoxyphenyl)thiazol-4-yl]pyridine-2-carboxylic Acid (19f). A mixture of **18b** (49 g, 127 mmol) and 10% NaOH (100 mL) in EtOH (1.4 l) was refluxed for 4 h. Much of the solvent was removed and the residue was partitioned between hot H_2O and EtOAc. The aqueous layer was acidified with 10% HCl and extracted with EtOAc, which was quickly washed with saturated aqueous NaCl and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was recrystallized from EtOAc to afford **19f** (43.3 g, 91.3%) as a white powder: mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, t, J = 6.9 Hz), 1.41 (3H, t, J = 6.9 Hz), 4.13 (2H, q, J = 6.9 Hz), 4.17 (2H, q, J = 6.9 Hz), 7.11 (1H, d, J = 8.4 Hz), 7.50–7.70 (2H, m), 8.04 (1H, dd, J = 1.2, 7.7 Hz), 8.15 (1H, t, J = 7.7 Hz), 8.42 (1H, dd, J = 1.2, 7.7 Hz), 8.49 (1H, s). Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

Compounds **19a–e** were prepared from corresponding esters, followed by hydrolysis as described for the preparation of compound **19f**.

Superoxide Anion Generation Assay.[16] Superoxide anion concentration was determined by the SOD inhibitable ferricytochrome *c* reduction assay. Isolated neutrophil[15] suspension was diluted with Hepes–Hanks solution (15 mM Hepes, 137 mM NaCl, 5.4 mM KCl, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 0.4 mM $MgSO_4$, 0.5 mM $MgCl_2$, 1.3 mM $CaCl_2$, and 5.6 mM glucose; the pH was adjusted to 7.4 with 2 N NaOH) to 1×10^7 cell/mL just before assay. Neutrophils (1×10^6 cell/mL) were preincubated at 37 °C in microtube (Iwaki glass, 1.5 mL) for 20 min with or without OPC-compounds. Cytochalasin B (1 μg/mL of final concentration) and ferricytochrome *c* (106 μM of final concentration) were added to neutrophil suspension at 10 and 4 min before the start of the reaction respectively. The reaction was initiated by the addition of stimulant, and after an adquate interval (this interval was dependent on the stimulus to get linearity), 1 mM *N*-ethylmaleimide was added to terminate the reaction, the mixture was centrifuged at 3000 rpm × 10 min to precipitate the cells, and the absorbance was measured at 550 nm by a spectrophotometer (JASCO UVIDEC-340). To detect superoxide specifically, the absorbance without SOD was subtracted from that with 25 μg/mL SOD.

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