# Thromboxane $A_2$ Synthetase Inhibitors with Histamine $H_1$-Blocking Activity: Synthesis and Evaluation of a New Series of Indole Derivatives[1)]

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**A novel series of N-substituted 3-(1*H*-imidazol-1-ylmethyl)indole carboxylic acid derivatives were prepared and evaluated for thromboxane $A_2$ ($TXA_2$) synthetase-inhibitory and histaminergic $H_1$-blocking activity. Among the compounds synthesized, indole-6-carboxylic acid derivatives showed higher activities than the other positional isomers of carboxylic acid. 1-[3-(4-Benzhydryl-1-piperazinyl)propyl]-3-(1*H*-imidazol-1-ylmethyl)-1*H*-indole-6-carboxylic acid (12) had the strongest thromboxane synthetase inhibitory activity ($IC_{50} = 5 \times 10^{-8}$ M) and $H_1$-blocking activity ($IC_{50} = 8 \times 10^{-9}$ M).**

**Key words** $TXA_2$ synthetase inhibitor; $H_1$-blocker; bronchoconstriction; structure–activity relationship; KY-234

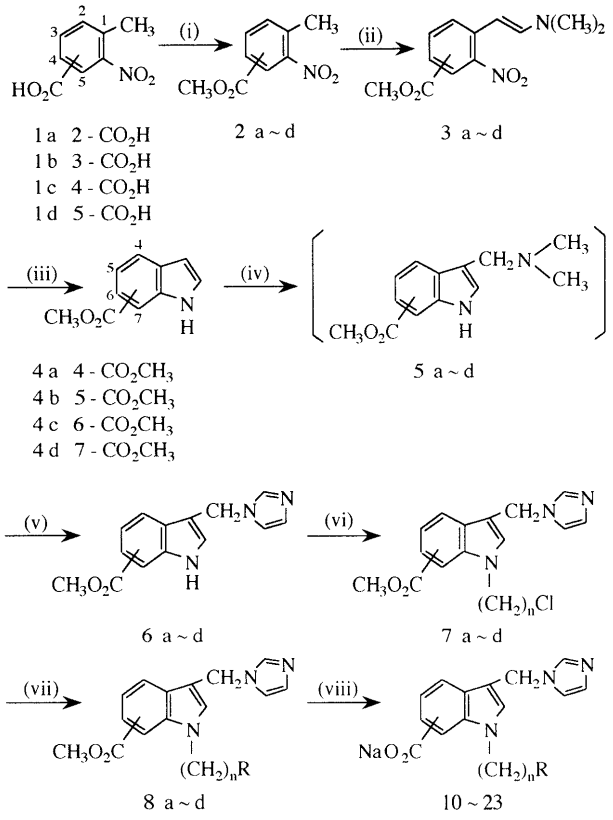
Thromboxane $A_2$ ($TXA_2$) and histamine are important chemical mediators involved in asthma. Ozagrel, a $TXA_2$ synthetase inhibitor,[2)] and terfenadine, a non-sedative $H_1$-blocker,[3)] have been used in the treatment of asthma. We hypothesized that a compound with both $TXA_2$ synthetase-inhibitory and $H_1$-blocking activities would provide more effective protection against asthma than a simple $TXA_2$ synthetase inhibitor or $H_1$-blocker. We have therefore synthesized a novel series of indole derivatives with substituents which might be suitable for $TXA_2$ synthetase inhibitors[4)] and histamine $H_1$-blockers.[5)] N-Substituted 3-(1*H*-imidazol-1-ylmethyl)indole (**9**) showed a weak inhibitory effect on arachidonate-induced platelet aggregation, which is $TXA_2$-dependent[6)] and histamine-induced contraction of guinea pig trachea, which is mediated by $H_1$-receptors.[7)] To enhance both activities, a carboxylic acid moiety was introduced into the indole ring at the 4, 5, 6 or 7 position of **9** and various substituents at the $N_1$-position. Among these compounds, 1-[3-(4-benzhydryl-1-piperazinyl)propyl]-3-(1*H*-imidazol-1-ylmethyl)-1*H*-indole-6-carboxylic acid (**12**) had the strongest $TXA_2$ synthetase-inhibitory and $H_1$-blocking activities. Herein, the synthesis, structure–activity relationship and pharmacological evaluation of this novel series of indole derivatives are described.

## Chemistry

Chart 1 shows the general synthetic pathway for preparation of the target compounds. The indole-carboxylic acid methylester (**4**) was prepared according to the Leimgruber–Batcho indole synthesis.[8)] The methylester (**2**) was prepared by acid-catalyzed esterification of the corresponding methyl-nitrobenzoic acid (**1**) in MeOH, and reaction of **2** with *N*,*N*-dimethylformamide dimethylacetal in DMF at 100 °C afforded **3**, which was converted to the indole methylester (**4**) by catalytic reduction using 5% Pd–C. Compound **6** was prepared from a Mannich base by refluxing with imidazole in xylene.[4)] The Mannich base (**5**) was obtained by treatment of the corresponding derivative (**4**) with 35% aqueous HCHO and 50% aqueous $HN(CH_3)_2$ in AcOH–dioxane at 20 °C. Treatment of **6** with NaH in DMF followed by dihalogenoalkane gave the N-halogenoalkyl-indole (**7**) and condensation of appropriate amines with **7** afforded **8**. The carboxylic acids (**10**—**23**) were prepared by base hydrolysis of the esters (**8**).

## Pharmacological Results and Discussion

In the present study, the inhibitory activity of derivatives towards $TXA_2$ synthetase and $H_1$-receptors was estimated




(i) HCl, $CH_3OH$, 80°, 6h ; (ii) N,N - dimethylformamide dimethyl acetal, DMF, 100°, 6.5h
(iii) $H_2$, Pd/C; (iv) HCHO aq, $HN(CH_3)_2$ aq, dioxane - AcOH;
(v) imidazole, xylene, 120°, 1h;
(vi) NaH, $Br(CH_2)nCl$, DMF, RT, 12h; (vii) appropriate amine, $K_2CO_3$, KI, DMF; (viii) 2N-NaOH, MeOH, 80°, 2h

Chart 1

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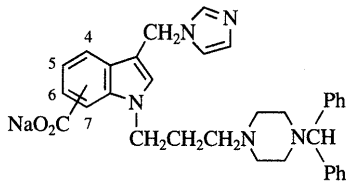
in terms of the effect on arachidonate-induced, $TXA_2$-dependent aggregation of rabbit platelets and histamine-induced, $H_1$-mediated contraction of guinea pig trachea. Tables 1 and 2 summarize the results. Ozagrel ($10^{-6}$—$10^{-4}$ M), a known $TXA_2$ synthetase inhibitor, concentration-dependently inhibited the arachidonate-induced aggregation, but did not affect the histamine-induced contraction of tracheal preparations. None of the compounds synthesized, or ozagrel, had any effect on ADP-induced, $TXA_2$-independent platelet aggregation. Pyrilamine ($10^{-9}$—$10^{-7}$ M), an $H_1$-blocker, concentration-dependently relaxed the tracheal preparations contracted with histamine. Cimetidine ($10^{-5}$ M), an $H_2$-blocker, failed to affect the histamine-induced contraction.

Introduction of a carboxylic acid moiety at the 4-position (**10**) or 5-position (**11**) of the indole ring decreased the inhibitory effect on arachidonate-induced platelet aggregation by more than 100-fold and its introduction at the 7-position (**13**) decreased the effect by 5-fold. In contrast, the isomer with COOH at the 6-position (**12**) had about 10-fold stronger activity than **9**. On the other hand, all the carboxylic acid isomers (**10**—**13**) had stronger histaminergic $H_1$-blocking activity than **9**, and among them **12** was the most potent. These findings revealed that the position of the carboxy moiety is important for both activities, and the 6-position of the indole is optimum. In the next set of experiments, we introduced various possible $H_1$-blocking substituents onto the $N_1$ position of 3-(1*H*-imidazol-1-ylmethyl)-1*H*-indole-6-carboxylic acid. As shown in Table 2, the non N-substituted compound (**14**) had little effect on the arachidonate-induced aggregation or $H_1$-mediated tracheal contraction. Introduction of a simple alkyl chain $C_3H_7$– (**15**) markedly enhanced only the inhibitory effect on arachidonate-induced platelet aggregation, indicating the importance of the side chain at the $N_1$-position for $TXA_2$ synthetase inhibition. Imidazole, carboxylic acid and the $N_1$-substituent of **15** may interact with three sites of the enzyme, as has been postulated for $TXA_2$ synthetase inhibitors such as CV-4151 and Y-20811.[9] Next, possible $H_1$-blocking substituents were introduced onto the $N_1$-position. Both $TXA_2$ synthetase-inhibitory and $H_1$-blocking activities varied with the substituents. As regards the inhibitory effect on arachidonate-induced platelet aggregation,

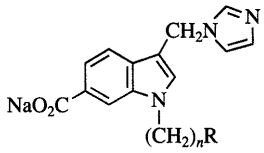
Table 1. Structures and Pharmacological Results

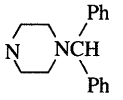
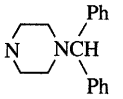
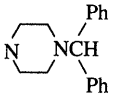
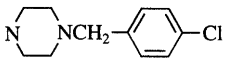
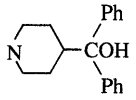
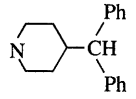
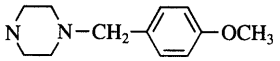
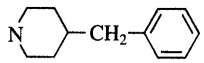
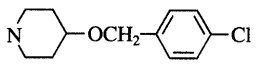


| No. | Position of $CO_2H$ | Inhibitory effect on AIPA[a] $IC_{25}$ ($\times 10^{-6}$ M) | Inhibitory effect on HIC[b] $IC_{50}$ ($\times 10^{-8}$ M) |
|---|---|---|---|
| **9** | None | 6.4 | 57.0 |
| **10** | 4 | >100 | 4.7 |
| **11** | 5 | >100 | 5.3 |
| **12** | 6 | 1.0 | 0.8 |
| **13** | 7 | 32.0 | 4.3 |
| Ozagrel | | 3.5 | >100 |
| Pyrilamine | | >100 | 0.3 |
| Cimetidine | | >100 | >100 |

*a*) AIPA: arachidonate-induced aggregation of rabbit platelets. *b*) HIC: histamine-induced contraction of guinea pig trachea.

Table 2. Structures and Pharmacological Results



| No. | *n* | R | Inhibitory effect on AIPA,[a] $IC_{25}$ ($\times 10^{-6}$ M) | Inhibitory effect on HIC,[b] $IC_{50}$ ($\times 10^{-8}$ M) |
|---|---|---|---|---|
| **14** | 0 | H | >100 | >100 |
| **15** | 3 | H | 6.5 | >100 |
| **16** | 2 |  | 2.5 | 1.5 |
| **12** | 3 |  | 1.0 | 0.8 |
| **17** | 4 |  | 5.4 | 2.2 |
| **18** | 3 |  | 4.0 | 9.8 |
| **19** | 3 |  | 14.4 | 1.8 |
| **20** | 3 |  | 2.1 | 6.9 |
| **21** | 3 |  | 20.4 | 130.0 |
| **22** | 3 |  | 2.1 | 6.9 |
| **23** | 3 |  | 1.1 | 12.0 |
| Ozagrel | | | 3.5 | >100 |
| Pyrilamine | | | >100 | 0.3 |
| Cimetidine | | | >100 | >100 |

*a*) AIPA: arachidonate-induced aggregation of rabbit platelets. *b*) HIC: histamine-induced contraction of guinea pig trachea.

Table 3. Biological Activities of **12** (KY-234)

| | $TXA_2$ production[a] | Bronchoconstriction[b] | | |
|---|---|---|---|---|
| | | $LTD_4$ | Histamine | Antigen |
| | $IC_{50}$ ($\times 10^{-7}$ M) | $IC_{50}$ (mg/kg, *p.o.*) | | |
| **12** (KY-234) | 0.5 | 6.0 | 2.5 | 6.3 |
| Terfenafdine | N.T. | >30 | 0.5 | 4.6 |
| Ozagrel | 5.0 | 7.1 | >30 | >30 |

*a*) Inhibitory effect on $TXA_2$ production in rabbit platelets. *b*) Inhibitory effect on bronchoconstriction induced by $LTD_4$, histamine or antigen in anesthetized guinea pigs.

Table 4. Physical Data for 3-(1*H*-Imidazol-1-ylmethyl)indole Derivatives

| No. | mp (°C) | Formula[a] | $^1$H-NMR (DMSO-$d_6$, $\delta$) |
|---|---|---|---|
| **6a** | 187—188 | $C_{14}H_{13}N_3O_2$ | 3.81 (3H, s), 5.47 (2H, s), 6.82 (1H, s), 6.97 (1H, s), 7.20 (1H, t), 7.49 (1H, s), 7.51 (1H, d), 7.64 (1H, d), 7.64 (1H, d), 11.60 (1H, br) |
| **6b** | 185—186 | $C_{14}H_{13}N_3O_2$ | 3.82 (3H, s), 5.40 (2H, s), 6.85 (1H, s), 7.12 (1H, s), 7.30—7.90 (4H, m), 8.20 (1H, s), 11.20 (1H, br) |
| **6c** | 187—189 | $C_{14}H_{13}N_3O_2$ | 3.84 (3H, s), 5.34 (2H, s), 6.83 (1H, s), 7.16 (1H, s), 7.40—7.90 (4H, m), 8.05 (1H, s), 11.50 (1H, br) |
| **6d** | 165—167 | $C_{14}H_{13}N_3O_2$ | 3.93 (3H, s), 5.36 (2H, s), 6.84 (1H, s), 7.00—7.30 (2H, m), 7.54 (1H, d, $J$=2.4 Hz), 7.70—8.00 (3H, m), 11.10 (1H, br) |
| **9** | Oil | $C_{32}H_{35}N_5$ | 1.70—2.50 (12H, m), 4.14 (2H, t), 4.27 (1H, s), 5.28 (2H, s), 6.81 (1H, s), 6.90—7.80 (16H, m), 8.30 (1H, s) |
| **10** | 120—122 | $C_{33}H_{34}N_5NaO_2$ | 1.70—2.50 (12H, m), 4.25 (2H, t), 4.27 (1H, s), 5.60 (2H, s), 6.72 (1H, s), 6.9—7.6 (15H, m), 7.65 (1H, s) |
| **11** | 128—130 | $C_{33}H_{34}N_5NaO_2$ | 1.70—2.50 (12H, m), 4.20 (2H, br t), 4.27 (1H, s), 5.33 (2H, s), 6.82 (1H, s), 7.00—7.60 (14H, m), 7.70 (1H, s), 8.15 (1H, s) |
| **12** | 130—133 | $C_{33}H_{34}N_5NaO_2$ | 1.70—2.50 (12H, m), 4.25 (2H, br t), 4.27 (1H, s), 5.30 (2H, s), 6.80 (1H, s), 7.10—7.80 (15H, m), 8.08 (1H, s) |
| **13** | 86—87 | $C_{33}H_{34}N_5NaO_2$ | 1.70—2.50 (12H, m), 4.37 (2H, br t), 4.24 (1H, s), 5.30 (2H, s), 6.80 (1H, s), 7.10—7.90 (16H, m) |
| **14** | 222—225 | $C_{13}H_{11}N_3NaO_2$ | 5.35 (2H, s), 6.86 (1H, s), 7.18 (1H, s), 7.50—8.00 (4H, m), 8.05 (1H, s), 11.50 (1H, br) |
| **15** | 179—181 | $C_{16}H_{17}N_3NaO_2$ | 0.85 (3H, t), 1.80 (2H, m), 4.19 (2H, t), 5.34 (2H, s), 6.86 (1H, s), 7.16 (1H, s), 7.50—8.00 (4H, m), 8.09 (1H, s) |
| **16** | 140—142 | $C_{32}H_{32}N_5NaO_2$ | 2.20—2.50 (10H, m), 4.35 (2H, br t), 4.40 (1H, s), 5.33 (2H, s), 6.85 (1H, s), 7.10—7.80 (15H, m), 8.10 (1H, s) |
| **17** | 100—103 | $C_{34}H_{36}N_5NaO_2$ | 1.40—2.50 (14H, m), 4.25 (2H, br t), 4.40 (1H, s), 5.33 (2H, s), 6.85 (1H, s), 7.10—7.80 (15H, m), 8.10 (1H, s) |
| **18** | 55—58 | $C_{27}H_{29}ClN_5NaO_2$ | 1.60—2.08 (12H, m), 3.43 (2H, s), 4.30 (2H, br t), 5.33 (2H, s), 6.85 (1H, s), 7.20 (1H, s), 7.30 (5H, br s), 7.50—7.90 (3H, m), 8.10 (1H, s) |
| **19** | 153—160 | $C_{34}H_{35}N_4NaO_3$ | 1.00—3.00 (13H, m), 4.30 (2H, br t), 4.50 (1H, m), 5.32 (2H, s), 6.85 (1H, s), 7.10—7.90 (15H, m), 8.10 (1H, s) |
| **20** | 135—140 | $C_{34}H_{35}N_4NaO_2$ | 1.00—1.60 (4H, m), 1.60—3.00 (9H, m), 3.56 (1H, d), 4.30 (2H, br t), 5.32 (2H, s), 6.85 (1H, s), 6.90—7.90 (15H, m), 8.10 (1H, s) |
| **21** | 68—70 | $C_{28}H_{32}N_5NaO_3$ | 1.80—2.60 (12H, m), 3.57 (2H, s), 3.72 (3H, s), 4.30 (2H, br t), 5.35 (2H, s), 6.85 (1H, s), 6.90 (2H, $A_2B_2$), 7.20 (1H, s), 7.25 (2H, $A_2B_2$), 7.60—8.00 (4H, m), 8.10 (1H, s) |
| **22** | 100—102 | $C_{28}H_{31}N_4NaO_2$ | 1.00—3.00 (15H, m), 4.30 (2H, br t), 5.35 (2H, s), 6.85 (1H, s), 7.25 (6H, br s), 7.40—8.90 (4H, m), 8.10 (1H, s) |
| **23** | 65—67 | $C_{28}H_{30}ClN_4NaO_3$ | 1.30—2.40 (10H, m), 2.50—2.90 (2H, m), 3.40 (1H, m), 4.30 (2H, br t), 4.46 (2H, br t), 5.32 (2H, s), 6.83 (1H, s), 7.14 (1H, s), 7.35 (4H, br s), 7.60—7.40 (4H, m), 8.09 (1H, s) |

*a*) All elemental analyses were within ±0.4% of calculated values.

**16**—**18**, **20**, **22** and **23** were slightly weaker, and **19** and **21** were markedly weaker than **12**. Interestingly, a structurally small difference between **19** and **20**, and between **18** and **21** resulted in a marked difference in the activity. As regards anti-histaminergic activity, **16**, **17** and **19** were slightly weaker and **18** and **20**—**23** were markedly weaker than **12**. These findings indicated that 1-[3-(4-benzhydryl-1-piperazinyl)alkyl]-3-(1*H*-imidazol-1-ylmethyl)-1*H*-indole-6-carboxylic acids have the strongest inhibitory activity on the arachidonate-induced platelet aggregation and the histamine-induced tracheal contraction. Comparison among **12**, **16** and **17** indicated that optimum length of the alkyl chain is that with $n=3$.

Thus, **12** was selected for further pharmacological evaluation and was named KY-234. As shown in Table 3, KY-234 inhibited the production of $TXA_2$, as determined by radioimmunoassay, about 10 times more strongly than ozagrel. KY-234 also inhibited the guinea pig bronchoconstriction induced by leukotriene $D_4$ ($LTD_4$), histamine and antigen at similar doses. Terfenadine inhibited only the histamine- and antigen-induced bronchospasm, and ozagrel attenuated only the $LTD_4$-induced constriction. KY-234 ($10^{-7}$—$10^{-4}$ M) did not affect the increase of the beating rate by histamine ($5\times10^{-6}$ M) in guinea pig right atrial preparations, while cimetidine reduced it concentration-dependently; the $IC_{50}$ value was about $3\times10^{-6}$ M.

In conclusion, KY-234, a novel indole derivative, inhibits experimental asthmatic bronchospasm *via* $TXA_2$ synthetase-inhibitory and $H_1$-blocking activities. KY-234 is a candidate anti-asthmatic drug.

## Experimental

Melting points were measured on a Yamato capillary melting-point apparatus without correction. Infrared (IR) spectra were recorded on a JASCO IR-810 spectrophotometer. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were taken with a Hitachi R-1900 instrument using tetramethylsilane as an internal standard. Chemical shifts are given in ppm. The following abbreviations are used; s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. Column chromatography was performed on 70—230 mesh silica gel from Merck.

**Methyl 4-Methyl-3-nitrobenzoate (2c)** Concentrated HCl (7.5 ml) was added to a solution of **1c** (15 g, 82.8 mmol) in MeOH (75 ml), and the reaction mixture was heated for 6 h at 80 °C. The solution was evaporated and the residue was carefully adjusted to slightly basic with saturated aqueous $NaHCO_3$ and extracted with AcOEt (150 ml). The AcOEt layer was washed with brine, dried ($Na_2SO_4$), filtered, and evaporated. The crystals were collected, washed with petroleum ether and dried to afford **2c** (14 g, 87%). mp 47.5—48.5 °C. IR (Nujol) $cm^{-1}$: 1730, 1620, 1530. $^1$H-NMR ($CDCl_3$) $\delta$: 2.69 (3H, s), 3.99 (3H, s), 7.44 (1H, d, $J$=8.0 Hz), 8.15 (1H, dd, $J$=2.0, 8.0 Hz), 8.59 (1H, d, $J$=2.0 Hz).

**Methyl Indole-6-carboxylate (4c)** A solution of **2c** (4.5 g, 23.1 mmol)

and *N*,*N*-dimethylformamide dimethylacetal (4.6 ml, 34.6 mmol) in DMF (11 ml) was stirred for 6.5 h at 100 °C. It was cooled, then $CH_2Cl_2$ (70 ml) and 5% Pd–C (0.5 g) were added and the mixture was stirred under a hydrogen atmosphere for 2 h. The reaction mixture was filtered, and $CH_2Cl_2$ (10 ml) and $H_2O$ (40 ml) were added to the filtrate. The organic solution was separated and washed with 2 N NaOH and brine, dried ($Na_2SO_4$), and evaporated. The residue was purified by chromatography (benzene) to give crystals **4c** (2.5 g, 62%). mp 79.5—80.0 °C. IR (Nujol) $cm^{-1}$: 1680, 1620, 1460. $^1$H-NMR ($CDCl_3$) $\delta$:3.93 (3H, s), 6.59 (1H, br t), 7.34 (1H, t, $J$=2.7 Hz), 7.65 (1H, d, $J$=6.0 Hz), 7.85 (1H, dd, $J$=1.5, 6.0 Hz), 8.18 (1H, d, $J$=1.5 Hz), 8.60 (1H, br).

**Methyl 3-(1*H*-Imidazol-1-ylmethyl)-1*H*-indole-6-carboxylate (6c)** A 50% aqueous $HN(CH_3)_2$ solution (31 ml, 0.30 mol) was added to a mixture of AcOH (65 ml), dioxane (65 ml) and 35% aqueous HCHO (24 ml, 0.30 mol) at 0—10 °C in an ice-water bath. The whole was stirred for 1 h at 8 °C, then the indole methylester **4c** (13 g, 74 mmol) was added and stirring was continued for 2 h at 0—10 °C. The reaction mixture was quenched with water (100 ml), carefully adjusted to slightly basic with 2 N NaOH, and extracted three times with $CHCl_3$. The combined organic extracts were washed with brine, dried ($Na_2SO_4$), filtered, and evaporated to give an oil **5c** as a crude product. The crude product **5c** in xylene (65 ml) was treated with imidazole (25 g, 0.37 mol). The reaction mixture was stirred for 1 h at 120 °C, then poured into ice-water (65 ml). The product was collected by filtration, washed with 99.5% EtOH, and recrystallized from 99.5% EtOH to give crystals **6c** (11 g, 58%). mp 187—189°C. IR (Nujol) $cm^{-1}$: 1700, 1625, 1550. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.85 (3H, s), 5.32 (2H, s), 6.86 (1H, s), 7.17 (1H, s), 7.62 (2H, s), 7.70 (1H, d, $J$=2.0 Hz), 7.77 (1H, s), 8.08 (1H, s), 11.55 (1H, br).

**Methyl 1-(3-Chloropropyl)-3-(1*H*-imidazol-1-ylmethyl)-1*H*-indole-6-carboxylate (7c)** A solution of **6c** (10 g, 39.2 mmol) in dry DMF (80 ml) was added to a suspension of sodium hydride (60% in mineral oil, 1.57 g, 39.2 mmol) in dry DMF (10 ml) with stirring in an ice-water bath. After 0.5 h, by which time gas evolution had ceased, bromochloropropane (3.9 ml, 39.2 mmol) was added. The reaction mixture was stirred at room temperature for 12 h, quenched with ice-water (100 ml) and extracted with AcOEt. The organic layer was washed with water (80 ml) and brine, and dried ($Na_2SO_4$). After filtration and concentration of filtrate *in vacuo*, the crude product was purified by chromatography ($CHCl_3$–MeOH, 20 : 1) to give crystals **7c** (10 g, 80%). mp 118—121 °C. IR (Nujol) $cm^{-1}$: 1700, 1620. $^1$H-NMR ($CDCl_3$) $\delta$: 2.05—2.50 (2H, m), 3.45 (2H, t, $J$=7.0 Hz), 3.92 (3H, s), 4.39 (2H, t, $J$=7.0 Hz), 5.28 (2H, s), 6.92 (1H, s), 7.05 (1H, s), 7.25 (1H, s), 7.45 (1H, d, $J$=7.0 Hz), 7.55 (1H, s), 7.81 (1H, dd, $J$=2.0, 7.0 Hz) 8.13 (1H, d, $J$=2.0 Hz).

**Methyl 1-[3-(4-Diphenylmethyl-1-piperazinyl)propyl]-3-(1*H*-imidazol-1-ylmethyl)-1*H*-indole-6-carboxylate (8c)** $K_2CO_3$ (828 mg, 6.0 mmol), KI (249 mg, 1.5 mmol) and diphenylmethylpiperazine (619 mg, 3.0 mmol) were added to a solution of **7c** (1.0 g, 3.0 mmol) in dry DMF (7 ml). The mixture was stirred for 12 h at 60 °C, poured into water and extracted with AcOEt. The organic layer was washed with brine, dried ($Na_2SO_4$) and evaporated. The residue was purified by chromatography ($CHCl_3$–MeOH, 50 : 1) to give crystals **8c** (1.3 g, 80%). mp 147—149 °C. IR (Nujol) $cm^{-1}$: 1740, 1670. $^1$H-NMR ($CDCl_3$) $\delta$: 1.70—2.60 (4H, m), 2.41 (8H, s), 3.90 (3H, s), 4.23 (1H, s), 4.30 (2H, m), 5.24 (2H, s), 6.86 (1H, s), 7.02 (1H, s), 7.00—7.50 (10H, m), 7.18 (1H, s), 7.40 (1H, d, $J$=7.0 Hz), 7.53 (1H, s), 7.76 (1H, dd, $J$=2.0, 7.0 Hz), 8.12 (1H, d, $J$=2.0 Hz).

**Sodium 1-[3-(4-Benzhydryl-1-piperazinyl)propyl]-3-(1*H*-imidazol-1-ylmethyl)-1*H*-indole-6-carboxylate (12)** A mixture of **8c** (8.0 g, 14.6 mmol), MeOH (8 ml) and 2 N NaOH (16 ml) was stirred for 2 h at 80 °C, then concentrated *in vacuo*. The residual solid was dissolved in EtOH, this solution was filtered and evaporated, and the residual crystals were recrystallized from EtOH to give **12** (5.0 g, 66%). mp 128—130 °C. IR (Nujol) $cm^{-1}$: 1660 , 1580. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.70—2.50 (12H, m), 4.25 (2H, br t), 4.27 (1H, s), 5.30 (2H, s), 6.80 (1H, s), 7.05—7.80 (15H, m), 8.08 (1H, s).

**Rabbit Platelet Aggregation (*in Vitro*)** Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from rabbit blood by centrifugation at 1000 rpm for 10 min and at 3000 rpm for 10 min at 4 °C, respectively. The platelet density of PRP was adjusted to $4 \times 10^5$ cells/ml. Platelet aggregation was measured with the NKK Hema Tracer (Niko Bioscience). The PRP was incubated with KY-234 ($3 \times 10^{-7}$—$10^{-4}$ M) or ozagrel ($3 \times 10^{-7}$—$10^{-4}$ M) at 37 °C for 1 min, followed by addition of arachidonic acid or adenosine 5′-diphosphate (ADP).

**Effect on Tracheal and Right Atrial Preparations** The isolated trachea of guinea pig was cut into 16 strips and 4 tracheal chain preparations were made by connecting every 4 strips. The right atrium was excised from the isolated heart. The tracheal and atrial preparations were suspended in a 10-ml organ bath containing Krebs–Henseleit solution under 1.0 and 0.5 g of passive tension, respectively. The nutrient solution was maintained at 37 °C, pH 7.4, and continuously aerated with 95% $O_2$–5% $CO_2$. Changes in tension were measured isometrically using a force-displacement transducer (Toyo Baldwin) and recorded on an ink-writing oscillograph (NEC San-Ei Instrument). The tracheal preparations were contracted with histamine ($5 \times 10^{-6}$ M), and test compounds were applied cumulatively. In the right atrium, histamine ($5 \times 10^{-6}$ M) was applied in the absence or presence of test drugs. The beating rate of the right atrium was calculated from the recordings of spontaneous contractions.

**Effect on $TXA_2$ Synthesis** The rabbit blood was collected from the carotid artery under anesthesia and mixed with acid citrate dextrose (ACD) solution (sodium citrate 85 mM, citric acid 64 mM and dextrose 110 mM). The PRP was obtained and the platelets were washed three times with Tyrode's solution. The platelets were finally suspended in Tyrode's solution at $10^8$ platelets/ml. In the presence of aspirin ($10^{-5}$ M), the platelet suspension was incubated at 37 °C for 10 min and then further incubated with $PGH_2$ for 30 min. $TXA_2$ produced was determined as $TXB_2$ by using a $TXB_2$[$^3$H]RIA kit.

**Effect on Bronchoconstriction** Under anesthesia, guinea pigs were ventilated at a constant volume of 4 ml at a frequency of 40 strokes/min with an artificial respirator (Igarashi Ika Kogyo). The cannula was inserted into the jugular vein, and D-tubocurarine (0.5 mg/kg) was intravenously injected. Airway resistance was measured by the modified overflow technique as reported previously.[10] Changes in insufflation pressure at a constant airflow were measured by a pressure transducer (Ugo Basile) and an amplifier (Nihon Denki Sanei) connected to the side-arm of the tracheal cannula. Bronchoconstriction was evoked by $LTD_4$ (0.15 mg/kg, i.v.) or histamine (10 mg/kg, i.v.) in unsensitized guinea pigs, and by BPO–BGG in passively sensitized animals. Test drugs were administered orally 1 h before the challenge with spasmogens. Passive sensitization of guinea pigs was carried out by intraperitoneal injection with anti-BPO–BGG guinea pig serum (0.2 ml/animal) 48 h before the experiment.[11]

## References and Notes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