Novel Antiasthmatic Agents with Dual Activities of Thromboxane A_2 Synthetase Inhibition and Bronchodilation. VI.[1] Indazole Derivatives

Masahisa YAMAGUCHI,* Noriaki MARUYAMA, Takaki KOGA, Kenshi KAMEI, Michitaka AKIMA, Toshio KUROKI, Masatomo HAMANA, and Nobuhiro OHI

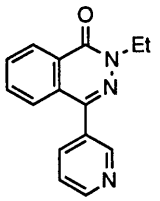
Fuji-gotemba Research Laboratories, Chugai Pharmaceutical Company, Ltd., 135, 1-chome Komakado, Gotemba-shi, Shizuoka 412, Japan. Received July 7, 1994; accepted September 12, 1994

Synthesis and pharmacological evaluation of novel indazole derivatives are described. These compounds were found to exhibit both thromboxane A_2 (TXA_2) synthetase-inhibitory and bronchodilatory activities. This observation supports the idea that the partial structure of the 3-pyridyl and phenyl groups with a methylene insertion is an important component for well-balanced activities.

Key words indazole derivative; TXA_2 synthetase inhibitor; bronchodilator; antiasthematic agent

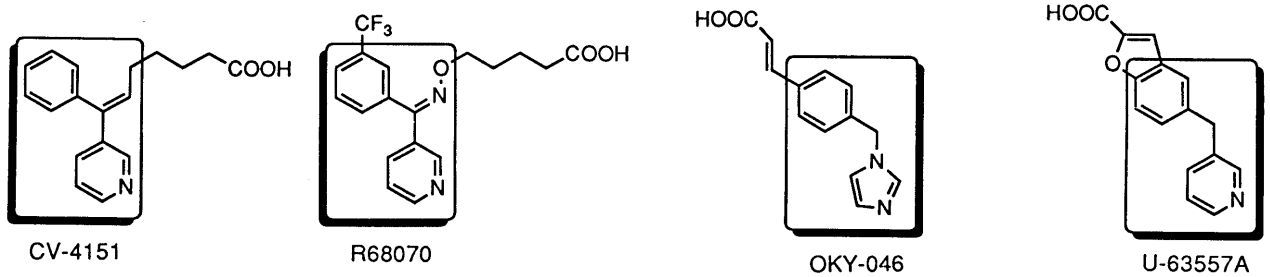
We have previously shown that some 2,4-disubstituted 1(2*H*)-phthalazinone derivatives, such as 2-ethyl-4-(3-pyridyl)-1(2*H*)-phthalazinone (**1**), have both thromboxane A_2 (TXA_2) synthetase-inhibitory and bronchodilatory activities (Fig. 1).[2b] In our studies on the phthalazinone system,[2] we have found that the essential components were the 3-pyridyl ring at the 4-position of **1** and the benzo moiety of the phthalazinone skeleton. In addition, the hydrophobicity of a compound was shown to have a marked influence on both activities. Taking account of the structures of some reported TXA_2 synthetase inhibitors such as CV-4151,[3] R68070,[4] OKY-046,[5] and U-63557A,[6] we noticed that a common component is the 3-pyridyl-methylene-benzo grouping and that our compounds are just like cyclization products of the above TXA_2 synthetase inhibitors (Fig. 2). As the mechanism of bronchodilation in our compounds remains to be elucidated, it is unclear whether the 3-pyridyl-methylene-benzo grouping has any role in bronchodilatory activity. We became interested in other ring systems, such as indazole bearing the above essential components, and carried out the synthesis of 1-(3-pyridyl)indazole derivatives.

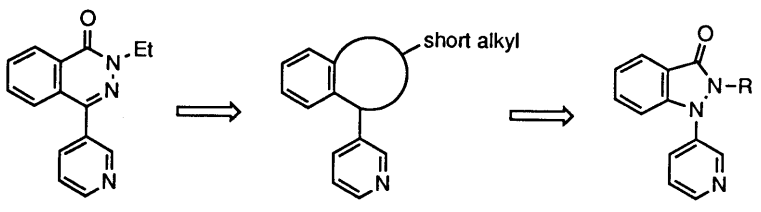
The desired indazole derivatives were synthesized according to the known procedure[7] with a slight modification. Reaction of 2-chlorobenzoic acid (**2**) with 3-aminopyridine in the presence of copper powder provided 2-(3-pyridylamino)benzoic acid (**3**), which was converted into the indazole (**4**) by *N*-nitrosation with nitrous acid followed by reduction with zinc/acetic acid.[8] Alkylation of **4** with alkyl halides in the presence of sodium hydride



1

Fig. 1. 2-Ethyl-4-(3-pyridyl)-1(2*H*)-phthalazinone

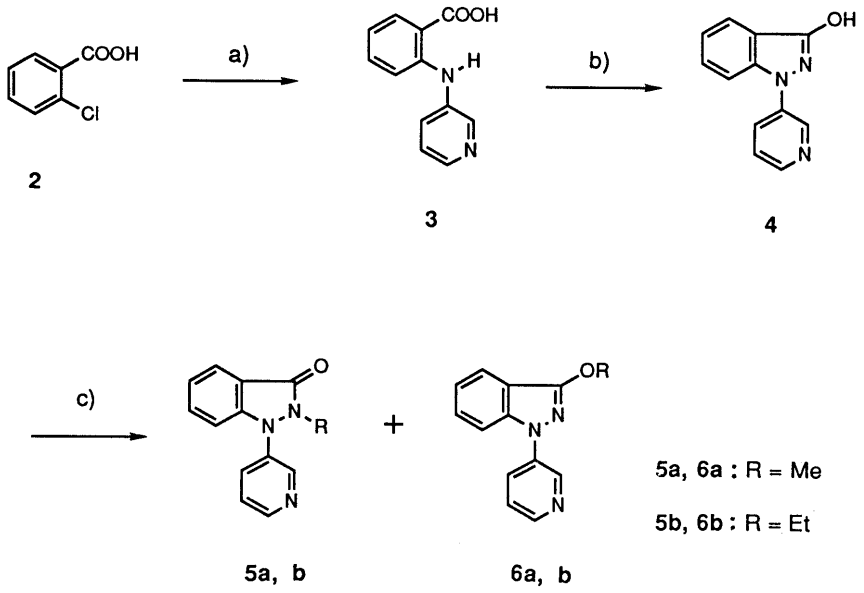




1

Fig. 2

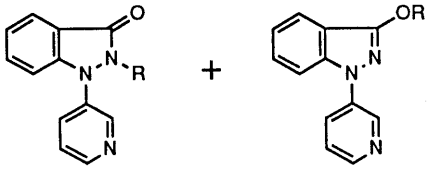
* To whom correspondence should be addressed.



a) 3-aminopyridine, K_2CO_3, Cu; b) (i) HNO_2; (ii) Zn, AcOH; c) MeI, NaH or EtBr, NaH.

Chart 1

TABLE I. TXA_2 Synthetase-Inhibitory and Bronchodilatory Activities



Compound	R	*Ex vivo* TXA_2 synthetase-inhibitory activity[a] Percent inhibition		*In vitro* bronchodilatory activity[b] $-\log[IC_{50}$ (M)]	
		3 mg/kg	10 mg/kg	Spontaneous	Histamine
1[c]		66	92	5.88	4.34
4		21	23	5.41	<4.0
5a	Me	22		<4.0	
5b	Et	24		<4.0	
6a	Me	49	70	5.02	
6b	Et	47	66	5.55	4.42
Aminophylline		0	0	4.33	3.50
OKY-046[d]		86	92	<3	<3

a) At 1 h after oral administration of test compounds ($n=4$). *b*) Concentration-activity curves were obtained with seven concentrations of test compounds, and IC_{50} values were calculated from the log curve ($n=2$). *c*) See reference 2*b*. *d*) See reference 5.

gave two compounds, 2-alkyl-1-(3-pyridyl)-1,2-dihydro-3*H*-indazol-3-one (**5a**; R = Me, **5b**; R = Et) and 3-alkoxy-1-(3-pyridyl)-1*H*-indazole (**6a**; R = Me, **6b**; R = Et). These compounds were readily separated by silica gel column chromatography (Chart 1).

TXA_2 synthetase-inhibitory and bronchodilatory activities were examined by using the previously described assay systems.[2] The *O*-alkyl derivatives (**6a** and **6b**) were shown to have significant activities, but the *N*-alkyl derivatives (**5a** and **5b**) were not so active (Table I). In particular, the ethoxy derivative **6b** was comparable in potency with the most effective compound **1**. The unsubstituted indazole (**4**) exhibited high bronchodilatory and moderate TXA_2 synthetase-inhibitory activities, consistent with those of the phthalazinone system. Although it is unclear why the *N*-alkyl derivatives were ineffective, these results support our hypothesis that ring systems other than the phthalazinone system having the 3-pyridyl-methylene-phenyl grouping can possess well-balanced activities.

In this study, we have found that the 3-pyridyl-methylene-phenyl grouping is an important component for well-balanced TXA_2 synthetase-inhibitory and bronchodilatory activities. Further chemical development and pharmacological evaluation of the above indazole derivatives are in progress.

Experimental

The melting points were measured with a Yanagimoto hot plate micro melting point apparatus and are uncorrected. The IR spectra were obtained with a Hitachi Model 270-30 infrared spectrometer. The ^{1}H-NMR spectra were taken with a Hitachi Model R-24B high-resolution magnetic resonance spectrometer (60 MHz) using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Shimadzu Model GCMS-QP1000 mass spectrometer and are reported as mass/charge ratio (relative intensity). organic extracts were dried over anhydrous sodium sulfate and concentrated in a rotry evaporator.

3-Hydroxy-1-(3-pyridyl)-1*H*-indazole (4) A suspension of 3-aminopyridine (12 g, 124 mmol), 2-chlorobenzoic acid (25 g, 124 mmol), K_2CO_3 (26 g, 186 mmol) and copper powder (0.3 g) in isoamyl alcohol (200 ml) was refluxed for 7 h. The mixture was concentrated, water was added and the whole was filtered. The filtrate was adjusted to pH 6 with concentrated HCl, and the resulting precipitates were collected, washed with EtOH, dried and recrystallized from EtOH to give 14.4 g (50%) of *N*-(3-pyridyl)anthranilic acid (**3**) as white crystals, mp 236—238 °C (lit.[7] 237—238 °C). **3** (8 g, 37 mmol) was dissolved in a solution of NaOH (1.7 g, 39 mmol) in water (55 ml), and $NaNO_2$ (3.6 g, 50 mmol) was added to the mixture. This solution was added dropwise to a solution of 3 N HCl (90 ml) at 5 °C and the mixture was stirred for 1 h at the same temperature. The resulting precipitates were collected, washed with water and dissolved in AcOH (60 ml). This solution was added to a mixture of zinc powder (7.8 g, 120 mmol) in water (20 ml), maintaining the temperature of the mixture between 10 and 20 °C. After having been stirred for 2 h, the mixture was heated to 80 °C and stirred for 30 min

at the same temperature. It was then filtered and the filtrate was poured into water. The resulting precipitates were collected, suspended in saturated $NaHCO_3$ and heated for a few minutes. The precipitates were collected, washed with water and dried to give 3.0 g (50%) of **4** as white crystals, mp 228—230 °C. IR (KBr): 1545, 1440 cm^{-1}. ^{1}H-NMR ($CDCl_3$–DMSO-d_6, 1 : 1) δ: 7.15—8.27 (6H, m), 8.40—8.58 (1H, m), 9.03—9.18 (1H, m). *Anal.* Calcd for $C_{12}H_9N_3O$: C, 68.25; H, 4.29; N, 19.89. Found: C, 68.12; H, 4.34; N, 19.81.

2-Methyl-1-(3-pyridyl)-2,3-dihydro-1*H*-indazol-3-one (5a) and 3-Methoxy-1-(3-pyridyl)-1*H*-indazole (6a) A suspension of **4** (1 g, 47 mmol), iodomethane (1 g, 70 mmol) and 60% NaH in oil (0.25 g, 61 mmol) in *N*,*N*-dimethylformamide (DMF) (30 ml) was stirred for 2 h at room temperature. The mixture was poured into water and extracted with $CHCl_3$. The extract was dried, concentrated and chromatographed on silica gel with $CHCl_3$–MeOH (100 : 1) to give 0.12 g (12%) of **5a** (more polar) and 0.36 g (36%) of **6a** (less polar). **5a** was obtained as white crystals, mp 113—115 °C ($CHCl_3$–hexane). IR (KBr): 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.28 (3H, s), 6.90—7.72 (5H, m), 7.75—8.10 (1H, m), 8.62—8.88 (2H, m). *Anal.* Calcd for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.16; H, 4.95; N, 18.66. **6a** was obtained as white crystals, mp 60—61 °C ($CHCl_3$–hexane). IR (KBr): 1545, 1445 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.17 (3H, s), 7.03—7.85 (5H, m), 7.90—8.18 (1H, m), 8.45—8.63 (1H, m), 9.05—9.23 (1H, m). *Anal.* Calcd for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.23; H, 4.94; N, 18.47.

In a similar manner, 2-ethyl-1-(3-pyridyl)-2,3-dihydro-1*H*-indazol-3-one (**5b**) and 3-ethoxy-1-(3-pyridyl)-1*H*-indazole (**6b**) were prepared. **5b** was obtained as white crystals, mp 150—151 °C ($CHCl_3$–hexane). IR (KBr): 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=7 Hz), 3.85 (2H, q, J=7 Hz), 6.95—7.84 (5H, m), 7.85—8.12 (1H, m), 8.63—8.86 (2H, m). *Anal.* Calcd for $C_{14}H_{13}N_3O$: C, 70.27; H, 5.48; N, 17.56. Found: C, 70.17; H, 5.55; N, 17.49. **6b** was obtained as white crystals, mp 83—84 °C ($CHCl_3$–hexane). IR (KBr): 1540, 1445 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, t, J=7 Hz), 4.53 (2H, q, J=7 Hz), 7.00—8.18 (6H, m), 8.42—8.62 (1H, m), 9.04—9.19 (1H, m). *Anal.* Calcd for $C_{14}H_{13}N_3O$: C, 70.27; H, 5.48; N, 17.56. Found: C, 70.27; H, 5.36; N, 17.44.

***Ex Vivo* Effect on Serum TXB_2 Concentration** Male SD rats (240—260 g) were starved for 20 h and dosed orally with test compounds (dissolved or suspended in 0.5% carboxymethylcellulose) or the vehicle. At 1 h after administration, the rats were anesthetized with ether, and blood (2 ml) was withdrawn from the heart and allowed to clot at 37 °C for 90 min. The clotted blood was centrifuged to obtain the serum, which was deproteinized with EtOH. The supernatant was stored at −20 °C. The serum TXB_2 concentration was measured with a TXB_2 radioimmunoassay kit (Amersham). The percent inhibition was calculated as the decrease in the serum TXB_2 concentration compared to each control group.

Relaxing Effect on Guinea Pig Isolated Tracheal Strips Guinea pig tracheal strips were suspended under isotonic conditions in oxygenated Krebs–Henseleit solution. Tension was allowed to develop spontaneously and resting tension was set at 1 g in the presence of aminophylline (10^{-3} M). Compounds were added in a cumulative fashion up to a maximum concentration of 100 μM and the relaxing effect was calculated as a percentage of the relaxation induced by aminophylline (10^{-3} M) added at the end of the experiment. The IC_{50} value of each compound was the concentration which produced 50% of the response to aminophylline as measured from the concentration–response curve, and values are generally (apart from compounds which had IC_{50} values of > 100 μM) the mean of three or more determinations. Each IC_{50} value is expressed as a negative logarithm.

Acknowledgment We wish to thank Dr. Y. Takeda and Dr. T. Yamazaki for their many useful suggestions.

References

1) M. Yamaguchi, N. Maruyama, T. Koga, K. Kamei, M. Akima, T. Kuroki, M. Hamana, N. Ohi, Novel antiasthmatic agents with dual activities of thromboxane A_2 synthetase inhibition and bronchodilation V. Thienopyridazinone derivatives. *Chem. Pharm. Bull.*, **43**, 236 (1994).
2) *a*) M. Yamaguchi, K. Kamei, T. Koga, M. Akima, T. Kuroki, N. Ohi, *J. Med. Chem.*, **36**, 4052 (1993); *b*) M. Yamaguchi, K. Kamei, T. Koga, M. Akima, N. Maruyama, T. Kuroki, N. Ohi, *ibid.*, **36**, 4061 (1993); *c*) M. Yamaguchi, T. Koga, K. Kamei, M. Akima, T. Kuroki, M. Hamana, N. Ohi, *Chem. Pharm. Bull.*, **42**, 1601 (1994); *d*) M. Yamaguchi, T. Koga, K. Kamei, M. Akima, N. Maruyama, T. Kuroki, M. Hamana, N. Ohi, *ibid.*, **42**, 1850 (1994).
3) K. Kato, S. Ohkawa, S. Terao, Z. Terashita, K. Nishikawa, *J. Med. Chem.*, **28**, 287 (1985).
4) F. De Clerck, J. Beetens, D. de Chaffoy de Courcelles, E. Freyne, P. A. J. Janssen, *Thromb. Haemost.*, **61**, 35 (1989).
5) K. Iizuka, K. Akahane, D. Momose, M. Nakazawa, T. Tanouchi, M. Kawamura, I. Ohyama, I. Kajiwara, Y. Iguchi, T. Okada, K. Taniguchi, T. Miyamoto, M. Hayashi, *J. Med. Chem.*, **24**, 1139 (1981).
6) J. L. Mehta, D. L. Lawson, W. W. Nichols, *Am. J. Physiol.*, **257**, 1240 (1989).
7) V. A. Petrow, *J. Chem. Soc.*, **1978**, 927.
8) L. Baiocchi, G. Corsi, G. Palazzo, *Synthesis*, **1978**, 633.