N-Phenylphthalimide-Type Cyclooxygenase (COX) Inhibitors Derived from Thalidomide: Substituent Effects on Subtype Selectivity

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Several *N*-substituted phenylphthalimide and phenylhomophthalimide derivatives with cyclooxygenase (COX)-inhibitory activity were prepared during structural development studies based on thalidomide as a lead compound. Substituent effects on the subtype selectivity were investigated.

Key Words Thalidomide; *N*-substituted phenylphthalimide; cyclooxygenase (COX); selectivity

Thalidomide (**1**) is a sedative and/or hypnotic drug, which was used from the late 1950's to the early 1960's, but was withdrawn from the market due to its severe teratogenicity.[1—3] In spite of this tragedy, thalidomide research was not halted, because of the drug's effectiveness against various kinds of diseases, including leprosy and AIDS.[2—4] Thalidomide was relaunched for the treatment of Hansen's disease in 1998 in the U.S., with special precautions for usage.

In order to explain the pleiotropic effects of thalidomide, we have postulated that thalidomide is a multi-target drug, and we have been engaged in structural development studies of thalidomide as a scaffold.[2,3,5—17] This systematic search has yielded tumor necrosis factor (TNF)-α production regulators,[2,3,5—7] androgen receptor antagonists,[2,3,8,9] peptidase inhibitors,[3,10—13] glucosidase inhibitors,[15,16] and thymidine phosphorylase inhibitors.[16] We suspected that cyclooxygenase (COX) might be another molecular target of thalidomide, since thalidomide is effective against colon and prostate cancers and possesses anti-angiogenic activity.[17,18]

Prostaglandin and thromboxane biosynthesis involves the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), a reaction catalyzed by the sequential actions of COX and prostaglandin endoperoxidase synthase (PGHS).[19] Three isoforms of COX (COX-1, COX-2, and COX-3) are known to date, of which COX-1, and COX-2 have been well investigated. COX-1 is constitutively expressed in many organs or tissues, while COX-2 is inducible with various stimuli. Overexpression of COX-2 has been detected in various tumors and its role in carcinogenesis and angiogenesis has been well documented.[20—22] Therefore, COX-2 is thought to be a promising therapeutic target for cancer.[20—22] Attempts have been made to apply COX-2 inhibitors, such as celecoxib and sulindac, for chemoprevention of various cancers, including colon and prostate cancers.[23,24]

Thalidomide suppresses lipopolysaccaride-induced expression of COX-2.[25,26] In addition, we have recently demonstrated that thalidomide directly inhibits COX-1/COX-2 with efficacy comparable to that of aspirin.[27] Much research has been done on the development of COX-2-selective inhibitors, including QSAR studies, but almost all of it deals with medium-sized molecules having a molecular weight of more than 300. Our earlier work on the COX-inhibiting activity of thalidomide afforded a new scaffold for small-molecular COX inhibitors (molecular weight of less than 300), which should be suitable for many kinds of structural development.

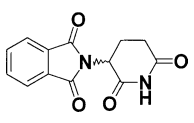

Fig. 1. Structure of Thalidomide

As a part of our continuing research directed toward the structural development of thalidomide as a multi-template for lead discovery, we report here novel COX inhibitors derived from thalidomide, focusing on COX-1- and COX-2-inhibitory activities.

Results and Discussion

The compounds discussed in this study were prepared by standard procedures, and the structures were confirmed by ^{1}H-NMR, mass spectrometry, and elemental analysis. Synthesis and chemical/physical data of compounds **2**, **5**, **6**, and **8** have already been reported.[5—8] Inhibitory activity of the compounds toward COX-1, and COX-2 was assayed by the use of a Colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman, No. 760111) according to the supplier's protocol.

Although the values differed from experiment to experiment, the results were basically reproducible, and typical sets of data are presented in Table 1. The assay was performed in triplicate, and repeated at least two times.

As can be seen from Table 1, aspirin exhibited moderate inhibitory activity towards both COX-1 (52% inhibition), and COX-2 (39% inhibition) at the concentration of 100 μM, under the experimental conditions used, suggesting that aspirin is a non-selective or slightly COX-1-selective inhibitor.

The position and the number of the methyl substituents located on the benzene ring in the present series of compound is important for the inhibitory activity on COX-1. *N*-Phenylphthalimide substituted at the 4-position of the benzene ring (**4**) is inactive. However, absence of substitution, and substitution of a methyl group at the 2- or 3-position resulted in apparent COX-1-inhibitory activity, although the potency was lower than that of aspirin at the same concentration. In contrast to the mono-substituted series, dimethylphenylphthalimide derivatives (**5**—**7**) did not show COX-1-inhibitory activity. These results suggest that the binding pocket of COX-1 for *N*-phenylphthalimide-type inhibitors is rather cramped, and physically larger derivatives, *i.e.*, dimethylphenylphthalimide derivatives (**5**—**7**) and *para*-methylphenylphthalimide (**4**), cannot be accommodated in the pocket.

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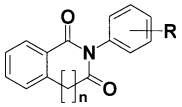
Table 1. COX-Inhibitory Activity of Compounds (**1**—**8**)



Compd.	*n*	R	% Inhibition[a)]	
			COX1	COX2
1	0	H	12.1	9.70
2	0	2-Me	12.1	19.0
3	0	3-Me	9.29	17.2
4	0	4-Me	0.400	24.0
5	0	2,6-diMe	ia[b)]	19.7
6	0	3,5-diMe	ia[b)]	9.70
7	0	2,3-diMe	ia[b)]	30.6
8	1	2,3-diMe	72.6	77.2
Aspirin			52.0	39.0

a) Compounds were screened for inhibitory activity on COX at the concentration of 100 μM. *b*) ia: inactive at the concentration of 100 μM.

On the other hand, for the COX-2-inhibitory activity, the opposite trend was seen in this series of compounds. The unsubstituted compound (**1**) and all the mono-methylphenylphthalimide derivatives (**2**—**4**) exhibited apparent COX-2-inhibitory activity, with the 4-methylated compound (**4**) being the most potent. Contrary to the result obtained with COX-1, *N*-dimethylphenylphthalimide derivatives (**5**—**7**) retained potent inhibitory activity, or showed moderate activity, towards COX-2 as compared to *N*-monosubstituted phenylphthalimides (with the exception of **6**). These results suggest that the binding pocket of COX-2 for *N*-phenylphthalimide-type inhibitors is larger than that of COX-1. The activity of the most potent compound, *i.e*., (**7**), was comparable to that of aspirin. Thus, compounds **4**, **5**, **6**, and **7** exhibited COX-2 selectivity, even though their structures are very simple. We thought it would be interesting to know whether this unique selectivity is derived from phthalimide structure, so we prepared a homologous analog, *i.e*., the homophthalimide derivative (**8**). *N*-2,3-Dimethylphenylhomophthalimide (**8**) exhibited almost the same inhibitory activity on both COX-1, and COX-2 (Table 1), and appears to be a potent (superior to aspirin) but non-selective COX inhibitor.

Previously, we reported that the electronic nature of the substituents introduced at the phthalimide moiety of methylthalidomide, the isoindolone moiety of *N*-substituted phenylisoindolone, and the isoindoline moiety of *N*-substituted phenylisoindoline dramatically changes the COX-1/2 selectivity of the compounds, depending on the substituent introduced.[27,28] Those reports and the results presented in this paper should be useful for the development of superior COX inhibitors with COX subtype selectivity.

Experimental

General Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within plus or minus 0.3% of the theoretical values. NMR spectra were recorded on a JEOL JNM-GX400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

2-(Substituted phenyl)-1*H*-isoindole-1,3-dione (General Method) A mixture of phthalic anhydride and substituted aniline was heated at 160 °C for 1 h. After cooling, the whole was dissolved in dichloromethane, dried with magnesium sulfate, and evaporated. The residue was recrystallized from a mixed solvent of *n*-hexane and dichloromethane to afford the desired compound as colorless crystals.

1: mp 195—198 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.97 (dd, J=5.6, 3.0 Hz, 2H), 7.80 (dd, J=5.6, 3.0 Hz, 2H), 7.52 (m, 2H), 7.44 (m, 3H); MS (FAB, M+H$^+$) 244; *Anal*. Calcd for $C_{14}H_9NO_2$: C, 75.33; H, 4.06; N, 6.27. Found: C, 75.22; H, 4.16; N, 6.55.

3: mp 170—172 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.96 (dd, J=5.1, 3.0 Hz, 2H), 7.79 (dd, J=5.1, 3.0 Hz, 2H), 7.39 (t, J=7.7 Hz, 1H), 7.23 (m, 3H), 2.42 (s, 3H); MS FAB, M+H$^+$) 238; *Anal*. Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.91; H, 4.76; N, 5.90.

4: mp 195—198 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.96 (dd, J=4.7, 3.0 Hz, 2H), 7.79 (dd, J=4.7, 3.0 Hz, 2H), 7.31 (m, 2H), 7.26 (m, 2H), 2.41 (s, 3H); MS (FAB, M+H$^+$) 238; *Anal*. Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.67; H, 4.76; N, 5.90.

7: mp 165—168 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.96 (dd, J=4.7, 3.0 Hz, 2H), 7.80 (dd, J=4.7, 3.0 Hz, 2H), 7.24 (m, 2H), 7.05 (d, J=7.3 Hz, 1H), 2.36 (s, 3H), 2.08 (s, 3H); MS (FAB, M+H$^+$) 252; *Anal*. $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.44; H, 5.45; N, 5.45.

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