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Thalidomide and Its Analogues as Cyclooxygenase Inhibitors

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Abstract—Thalidomide showed cyclooxygenase (COX)-1/2 inhibitory activity with a potency comparable to that of aspirin. Structural development studies of thalidomide resulted in potent COX-1/2 inhibitors, and COX-1-selective and COX-2-selective inhibitors.

Introduction

Cyclooxygenase (COX) is an enzyme which catalyzes the synthesis of prostaglandins from arachidonic acid, and is well-known as a target molecule of non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin.[1–3] There are two isoforms of COX. COX-1 is constitutively expressed in most tissues, whereas COX-2 is inducible. Overexpression of COX-2 has been detected in various tumors and its role in carcinogenesis and angiogenesis has been well-documented.[3–5] Consequently, COX-2 has been suggested to be an important pharmacological target for the prevention and treatment of cancer.[3–5] Attempts have been made to apply COX-2 inhibitors, including celexocib and sulindac, for chemoprevention of various cancers, including colon and prostate cancers.[6,7]

Thalidomide (**1**: Fig. 1) is a sedative/hypnotic drug which was withdrawn from the market because of its severe teratogenicity.[8–10] In spite of this, research into thalidomide was not halted, and the drug has been established to be effective for the treatment of various diseases, including leprosy, myeloma, AIDS, and so on.[9–11] The drug was approved in the United States for the treatment of leprosy in 1998, and clinical studies of its use for the treatment of various cancers, including myeloma, colon cancer, prostate tumor and breast cancer are on-going. The molecular mechanisms of the pharmacological actions elicited by thalidomide are not clear, but its tumor necrosis factor (TNF)-α production-inhibitory activity has been well-documented.[9–13]

We have demonstrated that the TNF-α production-regulating activity of thalidomide is bidirectional, and thalidomide is a multi-target drug.[9,10,14–25] We have been engaged in structural development studies of

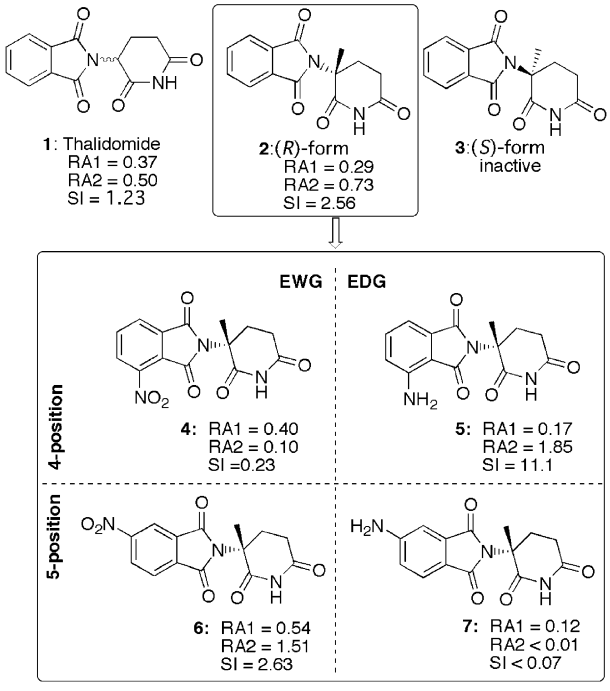

Figure 1. Effects of substituents on COX-inhibiting activities of thalidomide analogues.

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thalidomide, and have obtained TNF-α production regulators (including bidirectional ones and pure inhibitors and enhancers),[9,10,14–16] androgen antagonists,[9,10,17,18] peptidase inhibitors,[10,19–22] glucosidase inhibitors,[23,24] thymidine phosphorylase inhibitors,[25] and so on. We suspected that COX is another target molecule of thalidomide, because the drug is effective against colon and prostate cancers and possesses anti-angiogenic activity,[26,27] in which COX plays an important role. Though thalidomide is known to suppress lipopolysaccharide-induced expression of COX-2,[28,29] the direct effect of the drug on COX has not been established, to our knowledge. In this paper, we describe the COX-inhibiting activity of thalidomide, and structural development studies aimed at COX-inhibiting activity.

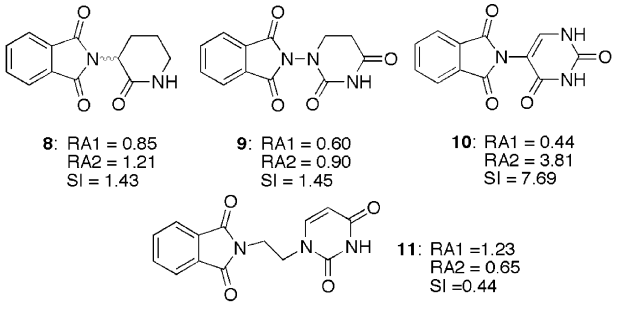
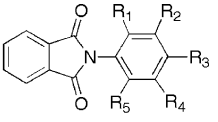


Figure 2. COX-inhibiting activities of some thalidomide analogues.



	R_1	R_2	R_3	R_4	R_5	RA1	RA2	SI
12:	H	H	H	H	H	<0.001	<0.001	-
13:	Me	H	H	H	H	<0.001	<0.001	-
14:	Me	H	H	H	Me	<0.001	<0.001	-
15:	H	Me	H	Me	H	7.4	13.2	2.14
16:	Me	Me	H	Me	H	0.33	1.18	4.05
17	Me	Me	H	Me	Me	0.20	0.67	4.16

Figure 3. COX-inhibiting activities of phenylphthalimide derivatives.

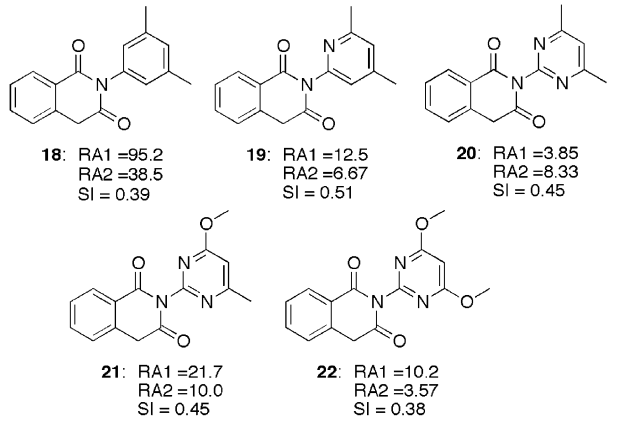


Figure 4. COX-inhibiting activities of homophenylphthalimide derivatives.

Results and Discussion

Compounds were prepared by our usual synthetic methods described previously,[14–25,30] and the structures were confirmed by NMR and mass spectrometry, and by appropriate analytical values. Briefly, 3′-methylthalidomide derivatives (**2**–**7**) were prepared by condensation of optically pure 3-amino-3-methylpiperidine-2-ones and substituted (or non-substituted) phthalic anhydrides, followed by imide formation and subsequent 3-*m*-chloroperoxybenzoic acid (MCPBA) oxidation.[30] Phthalimides (**10**–**17**) and homophthalimides (**18**–**22**) were prepared by condensation of phthalic anhydride or homophthalic anhydride, respectively, with appropriate amines.[14–25] The products were purified by silica gel column chromatography and recrystallization. The physico-chemical data of novel compounds (**18**–**22**) are given in References and Notes.[31–35]

Inhibitory activity of the compounds on COX-1 and COX-2 was assayed by the use of Colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman, No. 760111) according to the protocol recommended by the supplier. Although the IC_{50} values calculated from the experiments deviated from experiment to experiment, the results were basically reproducible, and typical sets of data are presented in Figures 1–4. In the assay system, the IC_{50} values of aspirin on COX-1 and COX-2 were determined to be 90–100 and 100–110 μM, suggesting that aspirin is a non-selective or slightly COX-1-selective inhibitor. The activities of compounds are presented as relative activities [RA values (RA1 and RA2, for COX- and COX-2 inhibiting activities, respectively)] defined as $IC_{50\ (aspirin)}/IC_{50\ (test\ compounds)}$, and the selectivity index (SI values) was defined as $IC_{50\ (COX\text{-}1)}/IC_{50\ (COX\text{-}2)}$. For compounds with weak activity, IC_{30} (for RA values of less than 0.3) or IC_{10} (for RA values of less than 0.1) values were used instead of IC_{50} values. The assay was performed in duplicate, and repeated at least three times.

As shown in Figure 1, thalidomide (**1**, racemate) possesses moderate non-selective or weakly COX-2-selective COX-inhibiting activity. Its non-racemizable *R*-form analogue, (*R*)-methylthalidomide (**2**) showed higher COX-2 selectivity with weaker COX-1-inhibiting and stronger COX-2-inhibiting activity compared to thalidomide (**1**). The corresponding *S*-form (**3**) was inactive. The effects of substituents introduced into the phthaloyl moiety of **2** were revealed to be bidirectional, being dependent on the position of introduction and the electronic nature of the substituent, that is (i) introduction of an electron-withdrawing nitro group at the 4-position (**4**) resulted in enhancement of COX-1-inhibiting activity and weakened the COX-2-inhibiting activity, resulting in a COX-1-selective inhibitor, while introduction of an electron-donating amino group at the same position (**5**) showed just the opposite effects, resulting in a COX-2-selective inhibitor, and (ii) introduction of a nitro group at the 5-position (**6**) enhanced both COX-1- and COX-2-inhibiting activity with higher potency for the latter, resulting in a COX-2-selective inhibitor, while introduction of an amino group at the

same position (**7**) lowered both COX-1- and COX-2-inhibiting activity with the higher potency for the latter, resulting in a COX-1-selective inhibitor.

The activities of some other thalidomide analogues (**8**–**11**) are shown in Figure 2. Among them, compound **10** is a COX-2-selective inhibitor which is more potent than aspirin. No clear-cut structure–activity relationships could be deduced at this stage.

The activities of phenylphthalimide derivatives are shown in Figure 3. Unsubstituted phenylphthalimide (**12**) was inactive. Methyl substituents on the *N*-phenyl group play critical roles in the COX-inhibiting activity of the compounds. The *m,m′*-dimethylphenyl analogue (**15**) showed the most potent activity, being 7.4 and 13.2 times more active than aspirin in COX-1 and COX-2-inhibiting activity, respectively. The structure–activity relationship of compounds **13**–**16** indicates that (i) introduction of methyl groups at the *meta* positions (**15**: potent, slightly COX-2-selective inhibitor), but not the *ortho* positions (**13**,**14**: both are inactive), is mandatory for potent COX-inhibiting activity, and (ii) additional introduction of methyl groups at the *ortho* positions (**16**,**17**: much weaker inhibitors than **15**) diminished the activity. Because the COX-2 selectivities of **16** and **17** are much higher than that of **15**, the COX-1-inhibiting activity seems to be more sensitive to this activity-diminishing effect of the *ortho* methyl group(s) compared to the COX-2-inhibiting activity. This result, as well as the previously described effect of the 3′-methyl group in compounds **2**–**7**, suggests the importance of steric hindrance and spatial structure around the nitrogen atom of the phthaloyl moiety.

Next we investigated the COX-inhibiting activity of homophthalimide analogues (**18**–**22**),[31–35] which were derived from our potent inhibitor **15**. As the aromatic substitutent to be introduced at the nitrogen atom of the phthaloyl moiety, we chose 3′,5′-disubstituted ones, because 3′,5′-dimethylphenylphthalimide (**15**) was the most potent inhibitor among the phenylphthalimide derivatives, **12**–**15** (Fig. 3).

Homophthalimide analogues generally showed very potent COX-inhibiting activity with RA values of 3.85–95.2, and they were all slightly COX-1-selective with SI values of 0.38–0.51. Among them, the *m,m′*-dimethylphenyl analogue (**18**) showed the most potent activity, being 95.2 times (IC_{50} of 0.98 μM) and 38.5 times (IC_{50} of 3.8 μM) more active than aspirin in COX-1- and COX-2-inhibiting activity, respectively. Insertion of hetero-atom(s) into the *N*-aryl group and/or changing aromatic methyl group(s) to methoxy group(s) generally lowered the activity without apparent modulation of the COX-1/COX-2 selectivity.

Conclusion

We found that thalidomide (**1**) possesses COX-1/2-inhibiting activity. Though the activity is weaker than that of aspirin, some of the pharmacological activities elicited by thalidomide, including anti-inflammatory and anti-colon cancer activities, might be attributed to the COX-inhibiting activity, at least in part. Structural modification to (*R*)-4-amino-3′-methylthalidomide (**5**) resulted in a highly COX-2-selective inhibitor with an SI value of 11.1. By structural development studies, the structurally simple compound **15**, *m,m′*-dimethylphenylphthalimide, was obtained as a potent and slightly COX-2-selective inhibitor. Based on this structure, a very potent COX inhibitor, *m,m′*-dimethylphenylhomophthalimide (**18**), was obtained. Further structural development aiming at improvement of the COX-1/COX-2 selectivity and pharmacological application studies are in progress.

Acknowledgements

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31. Compound **18**: mp 129–130 °C. ^{1}H NMR ($CDCl_3$/δ): 8.24 (1H, d, J=7.9 Hz), 7.63 (1H, dt, J=7.6, 1.2 Hz), 7.47 (1H, t, J=7.6 Hz), 7.34 (1H, d, J=7.6 Hz), 7.07 (1H, s), 6.82 (2H, s), 4.21 (2H, s), 2.36 (6H, s). m/z: 265 (M^+). Anal. calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.23; H, 6.37; N, 4.84.
32. Compound **19**: mp 184–186 °C. ^{1}H NMR($CDCl_3$/δ): 8.23 (1H, d, J=7.9 Hz), 7.63 (1H, dt, J=7.6, 1.2 Hz), 7.46 (1H, t, J=7.6 Hz), 7.33 (1H, d, J=7.6 Hz), 7.09 (1H, s), 6.91 (1H, s), 4.21 (2H, s), 2.56 (3H, s), 2.38 (3H, s). m/z: 266 (M^+). Anal. calcd for $C_{16}H_{14}N_2O_2$; C, 72.17; H, 5.30; N, 10.52. Found: C, 72.13; H, 5.53; N, 10.30.
33. Compound **20**: mp 251–253 °C. ^{1}H NMR($CDCl_3$/δ): 8.23 (1H, d, J=7.9 Hz), 7.63 (1H, dt, J=7.6, 1.2 Hz), 7.36 (1H, t, J=7.9 Hz), 7.33 (1H, d, J=7.6 Hz), 7.14 (1H, s), 4.21 (2H, s), 2.57 (6H, s). m/z: 267 (M^+). Anal. calcd for $C_{15}H_{13}N_3O_2$; C, 67.41; H, 4.90; N, 15.72. Found: C, 67.48; H, 4.96; N, 15.55.
34. Compound **21**: mp 217–218 °C. ^{1}H NMR($CDCl_3$/δ): 8.24 (1H, d, J=7.9 Hz), 7.64 (1H, dt, J=7.6, 1.5 Hz), 7.47 (1H, t, J=7.9 Hz), 7.34 (1H, d, J=7.6 Hz), 6.65 (1H, s), 4.21 (2H, s), 3.95 (3H, s), 2.52 (3H, s). m/z: 283 (M^+). HRMS (EI, M^+) $C_{15}H_{13}N_3O_3$, calcd for 283.096. Found 283.097.
35. Compound **22**: mp 224–226 °C. ^{1}H NMR($CDCl_3$/δ): 8.25 (1H, d, J=7.9 Hz), 7.64 (1H, dt, J=7.3, 1.5 Hz), 7.48 (1H, t, J=7.3 Hz), 7.34 (1H, d, J=7.6 Hz), 6.11 (1H, s), 4.21 (2H, s), 3.91 (6H, s). m/z: 299 (M^+). Anal. calcd for $C_{15}H_{13}N_3O_4$; C, 60.20; H, 4.38; N, 14.04. Found: C, 59.92; H, 4.44; N, 13.79.