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Inhibitory effects of 2-substituted-1-naphthol derivatives on cyclooxygenase I and II

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Abstract—Naphthol derivatives, 2-(3′-hydroxypropyl)-naphthalen-1-ol (**2**), 2-(3′-hydroxy-2′-methylpropyl)-naphthalen-1-ol (**3**) and 2-(3′-hydroxy-2′,2′-dimethylpropyl)-naphthalen-1-ol (**7**) were synthesized and already reported by our group. Therefore in this paper we described further synthesis of their ether derivatives, 3-(1-methoxy-naphthalen-2-yl)-propan-1-ol (**4**), 3-(1-methoxy-naphthalen-2-yl)-2methyl-propan-1-ol (**5**), 3-(1-methoxy-naphthalen-2-yl)-2,2-dimethyl-propan-1-ol (**8**), 2-(3-methoxy-propyl)-naphthalen-1-ol (**10**) and 2-(3-methoxy-2,2-dimethyl-propyl)-naphthalen-1-ol (**13**). Compounds **4**, **5** and **8** were prepared by methylation of compounds **2**, **3** and **7**, respectively while compounds **10** and **13** were prepared in good yield from naphthols **2** and **7**, respectively. When tested for inhibitory activity, five compounds (**2**, **3**, **7**, **10** and **13**) showed preferential inhibition of COX-2 over COX-1, while compounds **4**, **5** and **8** lacked inhibitory effect on either the COX-1 or COX-2 isozyme. The structure–activity relationships of these naphthols analyzed by docking experiments, indicated that the presence of hydroxyl group at C-1 position on the naphthalene nucleus enhanced the anti-inflammatory activity towards COX-2 via hydrogen bonding to the COX-2 Val 523 side chain. When this hydroxyl group was replaced by methoxy group, there was no inhibition. C-2′ Dimethyl substituents on the propyl chain also increased the inhibitory activity. All active compounds have the C-1 hydroxyl group aligned so as to form hydrogen bond with Val 523. The results provide a model for the binding of the naphthol derivatives to COX-2 and facilitate the design of more potent or selective analogs prior to synthesis.

1. Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and naproxen are a group of drugs that inhibit prostaglandin production via inhibition of the cyclooxygenase (COX) enzyme.[1,2] There are two isoforms of COX that is the constitutively induced COX-1, which is associated with maintaining homeostasis, and the inducible COX-2, which is normally not induced but plays an important role during inflammation.[3–5] Consequently, a newer generation of NSAIDs, which selectively inhibit COX-2 without affecting COX-1 activity, and thus which reduce harmful side effects, is desirable. Understanding the relationship of chemical structure and enzyme activity is crucial to selecting inhibitory compounds.

As part of our studies of the syntheses of antitumour, 1,2-naphthoquinone,[6] 1,4-naphthoquinone,[6] rhinacanthin and naphthoquinone ester derivatives,[7] the synthesis of naphthol derivatives was undertaken. The effects of 1-naphthol itself on enzymatic arachidonic acid oxidation have been previously reported by several workers.[8] Batt and co-workers described studies of naphthalen-1-ol, bearing carbon-linked substituents at the C-2 position, which are very potent inhibitors of 5-lipoxygenase (the enzyme catalyzes the first step in the oxidation of arachidonic acid to leukotrienes) and also inhibit cyclooxygenase. An example of compounds from this series is DuP 654 (2-benzyl-naphthalen-1-ol).[8] Therefore, our synthesized naphthols were evaluated

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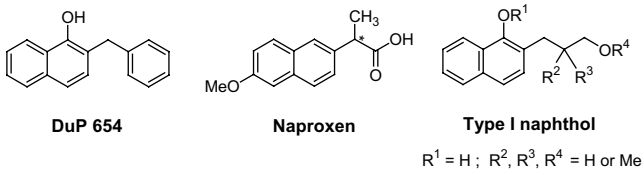


Figure 1. Structures of DuP 654, naproxen and naphthol.

for anti-inflammatory activity and it was found that our type I naphthol (Fig. 1) showed selective inhibition of COX-2 over COX-1. So molecular docking procedure of the tested naphthols was applied in order to establish relationships between different modes of binding and the activities of the compounds. We expect that the results could provide a model for binding of naphthol derivatives to COX-2 and assist the design of more potent or selective analogs prior to synthesis.

Here, we report our synthesized anti-inflammatory naphthol derivatives, which are 2-(3′-hydroxypropyl)-naphthalen-1-ol (**2**), 2-(3′-hydroxy-2′-methylpropyl)-naphthalen-1-ol (**3**), 2-(3′-hydroxy-2′,2′-dimethylpropyl)-naphthalen-1-ol (**7**), 2-(3′-methoxypropyl)-naphthalen-1-ol (**10**) and 2-(3′-methoxy-2′,2′-dimethylpropyl)-naphthalen-1-ol (**13**). These five naphthols can be prepared in several successive steps with significantly high yield in each step.[6,7] To demonstrate whether the compounds and their methyl naphthyl ether derivatives preferentially inhibit COX-2 isozyme over COX-1, we challenged murine COX-1 null and COX-2 null cell lines with these compounds and measured the reduction in the amount of prostaglandin E_2(PGE_2), which was used as an indicator of COX-2 and COX-1 inhibition. Their inhibitory effects on COX-1 and COX-2 were compared with a commercially available NSAID, naproxen, which has a naphthalene nucleus as a backbone. The biological structure–activity relationships (SARs) are discussed with respect to four features of the naphthol molecule: the nature of the C-2′ substituents, substitution on the naphthalene ring, the presence of the C-1 hydroxyl or methoxy group on the naphthalene ring and the presence of the hydroxyl group or methoxy group on the C-3′ position.

2. Results and discussion

2.1. Chemistry

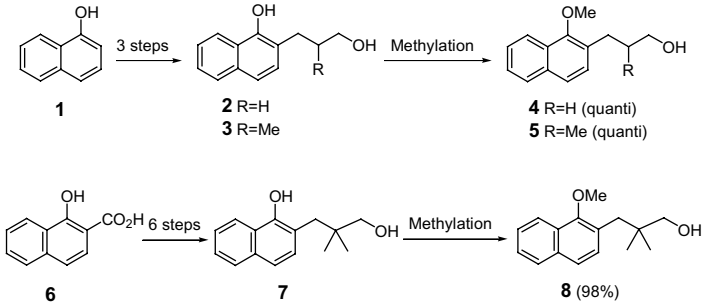
We have previously reported[6,7] the synthesis of naphthols **2** and **3** starting from naphthalen-1-ol (**1**) in three steps[6] as well as the synthesis of naphthol **7** starting from 1-hydroxy-2-naphthoic acid (**6**) in six steps.[7]

In this experiment, methylation of compounds **2** and **3** gave the methyl naphthyl ethers **4** and **5**, respectively in quantitative yield. Compounds **3** and **5** were obtained as racemic mixtures. Refluxing of naphthol **7** with methyl iodide in the presence of potassium carbonate gave methyl naphthyl ether **8** in 98% yield (Scheme 1).

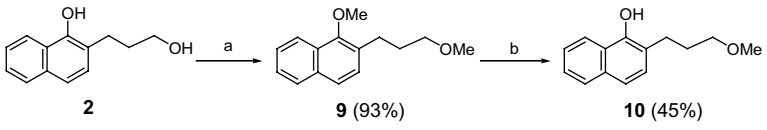
Naphthol **10** was prepared from naphthol **2** by methylation at both hydroxyl groups and then selective demethylation of the resulting methoxy-naphthalene **9** by using boron tribromide (Scheme 2). Naphthol **13** was synthesized from naphthol **7** in high yield. Selective protection of the naphthol function as benzyl naphthyl ether **11** followed by methylation of the primary hydroxy group afforded compound **12** in 94% yield. Debenzylation of the benzyl ether **12** provided naphthol **13** in 87% yield (Scheme 3).

2.2. Bioactivity

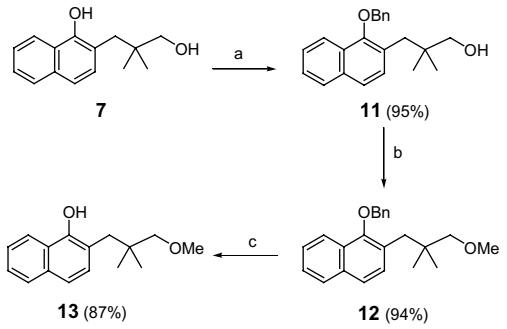
To test whether these compounds exhibited anti-inflammatory activities, we used the COX-1 deficient and COX-2 deficient murine fibroblast cell lines to investigate the relative potencies and selectivity of these compounds.[9] The reduction of PGE_2 produced in these two cell lines corresponds to the inhibition of COX-2 and COX-1 enzymes.[10] DMSO (0.1%) and aspirin were



Scheme 1. Synthesis of naphthols **2**, **3**, **7** and naphthyl ethers **4**, **5**, **8**.



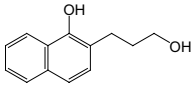
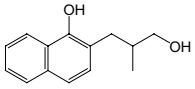
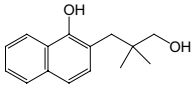
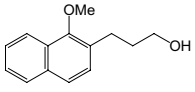
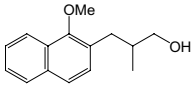
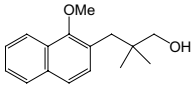
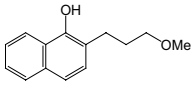
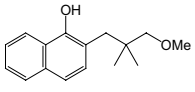
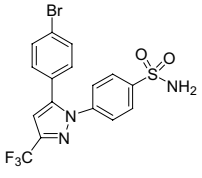
Scheme 2. Reagents and conditions: (a) MeI, NaH, reflux, 3 h; (b) BBr_3, CH_2Cl_2, 0 °C, 1 h.



Scheme 3. Reagents and conditions: (a) BnCl, K_2CO_3, acetone, reflux, 5 h; (b) NaH, MeI, THF, reflux, 5 h; (c) TMSCl, KI, CH_3CN, rt, 1 h.

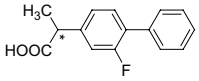
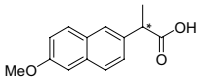
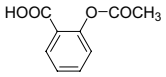
used as control for 100% COX activities and as positive control, respectively. Compounds **2** and **3** showed similar potency against COX-2 with IC_{50} values in the range of 4.2–4.6 μM while compound **7** was more potent against COX-2 with IC_{50} of 1.7 μM (Table 1). All these compounds inhibited COX-2 in a dose dependent manner (data not shown) and preferentially inhibited COX-2 over COX-1 as demonstrated by the lower COX-2 IC_{50} values. Their methyl ether derivatives, compounds **4**, **5** and **8**, showed no significant inhibition of either COX-1 or COX-2. Naphthalen-1-ol (**1**) showed approximately twofold selective inhibition of COX-2 over COX-1. Naproxen, an NSAID based on a naphthalene structure, was reported to slightly selectively inhibit COX-1 over

Table 1. Structure of naphthol compounds, the experimental activity (IC_{50} values) of compounds tested as inhibitors of COX-1 and COX-2 and their cytotoxicity against Vero cells

Compound	Structure	IC_{50} of COX-1 (μM)[a]	IC_{50} of COX-2 (μM)[a]	Ratio of COX-1/COX-2	IC_{50} of Vero cells (μM)
Naphthalen-1-ol (**1**)		0.97	0.40	2.4	215.0
2		93.9	4.2	22.4	>247.2
3		87.8	4.6	19.1	171.1
7		3.4	1.7	2	>217.1
4		Inactive	Inactive	—	—
5		Inactive	Inactive	—	—
8		Inactive	Inactive	—	—
10		15.7	0.25	62.8	>231.2
13		>40.9	4.1	>10.0	>204.6
SC-558		17.7	0.0093	—	—

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	IC_{50} of COX-1 (μM)[a]	IC_{50} of COX-2 (μM)[a]	Ratio of COX-1/COX-2	IC_{50} of Vero cells (μM)
Flurbiprofen		2.56	0.29	8.8	—
Naproxen		27.8	43.4	0.6	>217.1
Aspirin		77.7	321.9	0.2	—

[a] Values are averaged from two to four experiments.

COX-2 and inhibit COX activities in the gastric mucosa.[11,12] In our test system, naproxen also preferentially inhibited COX-1 over COX-2 to a slight degree (Fig. 7). Interestingly, both compounds **10** and **13** exhibited at least 10-fold selective inhibition of COX-2 over COX-1, suggesting an important role of the C-3′methoxy group. While COX-2 is a target of inhibition by NSAIDs, the harmful side effects such as gastric damage by currently used NSAIDs are associated with COX-1 inhibition.[12–14] When tested for cytotoxicity employing the colorimet- ric method modified from an anti-cancer screening method[15] with Vero cell line, compound **3** showed toxic effects to Vero cells similar to naphthalen-1-ol (**1**), while compounds **2**, **7**, **10**, **13** and naproxen are not toxic to Vero cells at concentrations as high as in the range of 217–247 μM. From our results of COX-1/COX-2 IC_{50} ratios and cytotoxicity IC_{50} values, it is conceivable that compounds **2**, **7** and **10** might confer less damaging side effects than naproxen. In all of our experiments, aspirin was employed as a nonselective COX-2 inhibitor with IC_{50} values for COX-1 and COX-2 of 77.7 and 321.9 μM, respectively.

2.3. Molecular docking

From the results of the inhibitory testing (Table 1), it was found that the presence of the hydroxyl group at the C-1 position of the naphthalene ring plays an important role in conferring inhibitory activity whereas the presence of the methoxy group gave no inhibition. Furthermore, the substituent at C-2′ of the propyl chain has some inhibitory effect against the COX-1 and COX-2. These results were verified by docking experiments as shown in Figure 4.

Docking outputs showed that compounds having naphthalene moieties have the ability to occupy the hydrophobic cavity (Fig. 2) formed by residues Phe 381, Tyr 385, Tyr 387, Phe 513 and Ser 530 in both COX-1 and COX-2 even in the case of naphthalen-1-ol as shown in Figure 6. This implied that affinity-binding to the hydrophobic pocket alone would not confer enzyme inhibition and selectivity. The data from enzyme assay experiments suggested that there was a relationship between the types of C-2′ substituents on the propyl chain

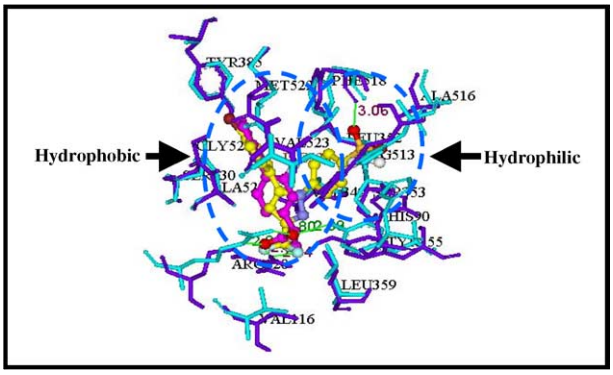


Figure 2. The COX-1 and COX-2 active sites are shown superimposed (COX-1, blue; COX-2, violet). Two inhibitors are seen: flurbiprofen (magenta), a nonselective inhibitor, and SC-558 (yellow), a COX-2-selective inhibitor. Note how the COX-2-selective inhibitor projects rightward into a side pocket that is not exploited by the nonselective inhibitor.

(compounds **2**, **3** and **7**) and COX-2 activity. We have also done docking experiment of the two stereoisomers of compound **3** and we have found that both isomers can occupy the binding site in the same orientation (Fig. 5).

The presence of hydroxyl group at C-1 on the naphthalene nucleus seemed to enhance COX-2 selectivity and inhibition, whereas the methoxy group (compounds **4**, **5** and **8**) at the same position reduced inhibitory activity as shown in Figure 4. Investigation on the COX-2 docked conformations revealed that all active compounds have the C-1 hydroxyl group aligned so as to form hydrogen bond with Val 523 (Figs. 4 and 8). Unlike the COX-2 selective inhibitory mechanism of SC-558,[16] the selectivity of this series was not contributed by the group bound to the hydrophilic pocket on the other side of Val 523. Also in the experiments, it was found that C-2′ dimethyl substituents increased inhibitory activity (compound **7** compared with **2**) in support to the finding of the other researchers.[8]

Almost all active compounds showed very low inhibition against COX-1. Comparison between the bound

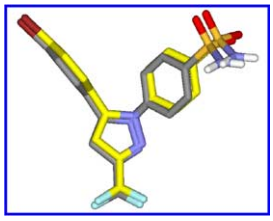


Figure 3. Conformational comparison of SC-558 from the crystal structure (grey) and that from AutoDock result (yellow).

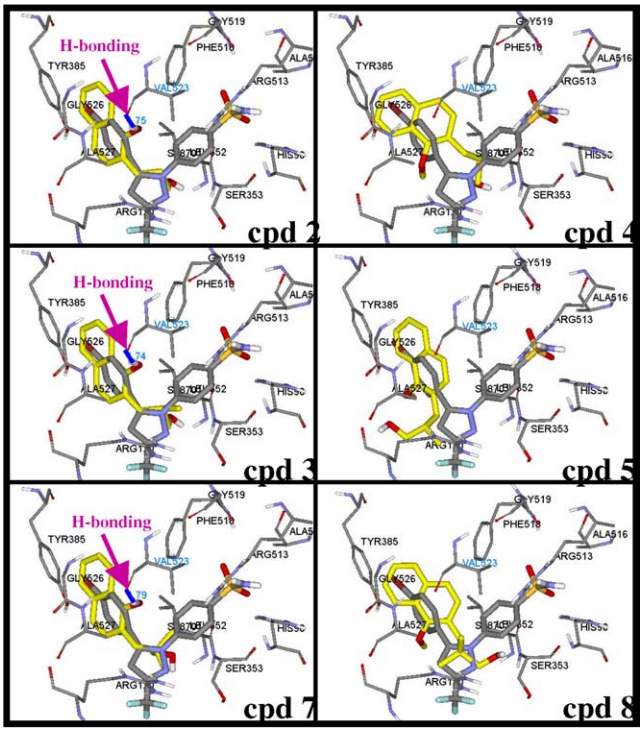


Figure 4. Comparison of the orientations of bound naphthol (inhibitor as obtained from AutoDock) in the binding site of COX-2. Val 523 is colored in blue. The hydrogen-bonding interactions are shown as blue lines. All tested compounds are shown in yellow: compounds **2**, **3**, **7**, **4**, **5** and **8**. The orientation of the naphthol group of active compounds (**2**, **3** and **7**) is upward to form hydrogen bonding while the methyl naphthyl ether group of inactive compounds (**4**, **5** and **8**) is oriented downward.

conformation of compound **2** in the binding site of COX-2 and COX-1 (Fig. 8) revealed that residue Ile 523 of COX-1 could not provide hydrogen bonding condition as good as that provided by Val 523 of COX-2. Table 1 showed that compound **10** has the same potent inhibitory effect as Flurbiprofen against COX-2 but compound **10** is much more selective for COX-2 (7 times) than Flurbiprofen. Compound **10** is much less active than SC-558.[17] The hydrophilic and hydrophobic moieties of compound **10** and Flurbiprofen are almost of the same configuration whereas compound SC-558,[16] which has bezenesulfonamide group as hydrophilic moiety and bromobenzene group as the hydrophobic portion has distinctly different configuration than compound **10**.

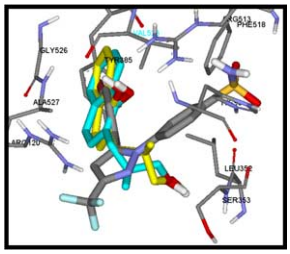


Figure 5. Superimposition of the docked conformation of the *R*-configuration (yellow) and the *S*-configuration (sky blue) of compound **3**, indicating similar binding orientation of both isomers in the COX-2 binding site.

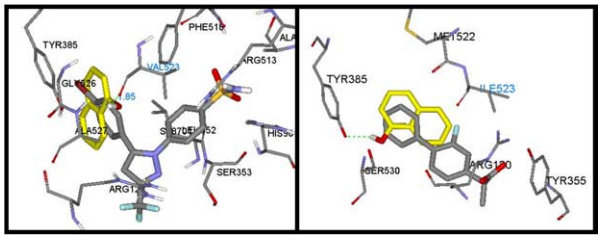


Figure 6. Possible orientations of naphthalen-1-ol in the binding site. (Left: COX-2, Right: COX-1).

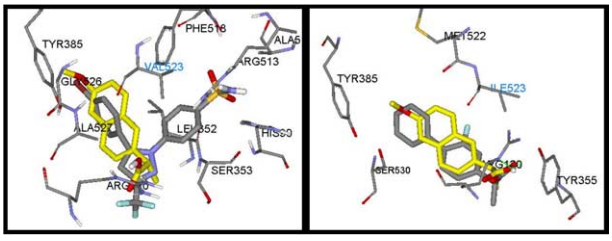


Figure 7. Comparison of the bound conformation of naproxen in the binding site. (Left: COX-2, Right: COX-1).

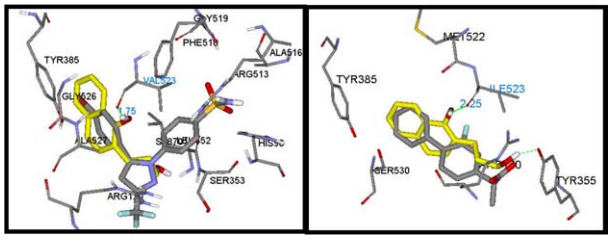


Figure 8. Comparison of the bound conformation of naphthol **2** in the binding site. (Left: COX-2, Right: COX-1).

3. Conclusions

The synthesized naphthols **2**, **3**, **7**, **10** and **13** showed inhibitory activity against cyclooxygenase. Most especially, compounds **2**, **3** and **10** showed significant activity with preferential COX-2 selectivity, while their methyl naphthyl ether derivatives (**4**, **5** and **8**) did not inhibit both COX-1 and COX-2 indicating the negative effect of an additional methyl group. Furthermore, all active

compounds showed no cytotoxic effect against Vero cell lines.

Molecular modeling experiments indicated no significant COX-1 inhibitory effect as a result of having some polarity (hydroxyl group) at the C-1 position of the naphthalene nucleus. Contrarily, the hydroxyl groups enhanced inhibition of COX-2 by hydrogen bonding to the COX-2 Val 523 side chain. Interestingly, naphthol **10** showed similar inhibitory effect as Flurbiprofen with both compounds occupying the binding site in the same orientation. However, COX-2 inhibition of our naphthols was lower than that of SC-558 since the compounds had no moiety comparable to the hydrophilic binding structure of SC-558 bound to the hydrophilic pocket of COX-2.

The structure–activity relationships of the naphthols analyzed by docking experiments, indicated that the C-1 hydroxyl group and C-2′ dimethyl substituents were the groups that enhanced the inhibition as shown in Table 1. The study showed that all active compounds formed hydrogen bonding with residue Val 523 of the enzyme of COX-2. In COX-1, no tested compound clearly gained the benefits of having hydrogen bond with Ile 523.

The results of this study provide a model for the binding of naphthol derivatives to COX-2. We are able to establish the relationships between two types of substituents (monomethyl and dimethyl) and the mode of interaction to the amino acid residue Val 523 of COX-2, which have not been reported before. This finding should be very useful to design more potent COX-2 selective analogs.

The synthesis of other naphthol derivatives and their anti-inflammatory evaluation as well as molecular docking by our group are being currently investigated.

4. Experimental

General remarks. Melting points were determined on a Fisher John apparatus and are uncorrected. The IR spectra were recorded on an FTIR Perkin–Elmer system 2000. Mass spectral data were obtained on the GCMS-QP-5050A. Nuclear magnetic resonance spectra were recorded at 400 MHz on a Brucker Advance DPX-400. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet. Column chromatography was performed with flash silica gel (Merck 9385).

4.1. General method for the methylation of naphthol (4 and 5)

A mixture of alcohol (0.5 mmol), methyl iodide (2.0 mmol) and potassium carbonate (1 mmol) in acetone (5 mL) was refluxed for 4 h. Then the reaction mixture was cooled to room temperature, filtered and washed with acetone. The filtrate was concentrated in vacuo, then diethyl ether was added and washed with water, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography.

4.1.1. 3-(1-Methoxy-naphthalen-2-yl)-propan-1-ol (4). Yield: quantitative; colourless oil; FTIR (neat, cm^{-1}): 3380 (OH), 1572 (C=C), 1085 (C–O), 1056 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.87 (m, 2H, CH_2), 2.20 (br s, 1H, OH), 2.87 (t, 2H, $J = 7.2$ Hz, CH_2), 3.53 (t, 2H, $J = 6.0$ Hz, OCH_2), 3.89 (s, 3H, OCH_3), 7.25 (d, 1H, $J = 8.4$ Hz, ArH), 7.43 (m, 2H, ArH), 7.53 (d, 1H, $J = 8.4$ Hz, ArH), 7.76 (d, 1H, $J = 7.7$ Hz, ArH), 8.02 (d, 1H, $J = 8.2$ Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.1, 33.9, 62.0, 62.8, 122.5, 125.1, 126.2, 126.7, 128.6, 128.9, 128.4, 130.2, 134.5, 154.0; EIMS (*m*/*z*, %): 216 (M^+, 100), 171 (79), 156 (40), 128 (60); Anal. Calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46. Found: C, 77.47; H, 7.64.

4.1.2. 3-(1-Methoxy-naphthalen-2-yl)-2methyl-propan-1-ol (5). Yield: quantitative; colourless oil; FTIR (neat, cm^{-1}): 3400 (OH), 1462 (C=C), 1258 (C–O), 1035 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.93 (d, 3H, $J = 6.8$ Hz, CH_3), 1.94 (m, 1H, CH), 2.32 (br s, 1H, OH), 2.65 and 2.81 ($2 \times$ m, 2×1H, CH_2), 3.30 (m, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 7.20 (d, 1H, $J = 8.4$ Hz, ArH), 7.40 (m, 2H, ArH), 7.49 (d, 1H, $J = 8.4$ Hz, ArH), 7.73 (d, 1H, $J = 7.8$ Hz, ArH), 7.98 (d, 1H, $J = 8.3$ Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.7 (CH_3), 33.5 (CH_2), 37.5 (CH), 62.8 (OCH_3), 66.9 (OCH_2), 122.6, 124.9, 126.2, 126.6, 128.6, 129.6 (CH arom), 128.3, 129.1, 134.5, 154.1 (C arom); EIMS (*m*/*z*, %): 230 (M^+, 50), 171 (100), 156 (44), 128 (69); Anal. Calcd for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.32; H, 7.92.

4.2. 3-(1-Methoxy-naphthalen-2-yl)-2,2-dimethyl-propan-1-ol (8)

Prepared by the general method for the methylation of naphthol. Yield: 98%; colourless oil; FTIR (neat, cm^{-1}): 3437 (OH), 1466 (C=C), 1080 (C–O), 1045 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.94 (s, 6H, $2 \times CH_3$), 2.71 (s, 2H, CH_2), 2.99 (s, 2H, OCH_2), 3.11 (br s, 1H, OH), 3.91 (s, 3H, OCH_3), 7.22 (d, 1H, $J = 8.4$ Hz, ArH), 7.46 (m, 2H, ArH), 7.53 (d, 1H, $J = 8.4$ Hz, ArH), 7.79 (m, 1H, ArH), 8.02 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.8 ($2 \times CH_3$), 38.2 (C), 38.7 (CH_2), 62.8 (OCH_3), 70.0 (OCH_2), 122.6, 124.4, 126.3, 126.7, 128.6, 131.1 (CH arom), 127.8, 128.0, 134.7, 154.3 (C arom); EIMS (*m*/*z*, %): 244 (M^+, 52), 171 (100), 156 (29), 128(47); Anal. Calcd for $C_{16}H_{20}O_2$: C, 78.65; H, 8.25. Found: C, 78.72, H, 8.09.

4.3. 1-Methoxy-2-(3-methoxy-propyl)-naphthalene (9)

To a stirred suspension of sodium hydride (0.14 g, 6 mmol) in dry THF (5 mL), a solution of naphthol **2** (0.2 g, 1 mmol) in dry THF (5 mL) was added dropwise. After refluxing for 3 h, the reaction mixture was cooled

to room temperature and quenched with water, then extracted with diethyl ether (3 × 50 mL). The combined organic phase was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 49:1 v/v hexane–ethyl acetate to afford the methyl ether product (93%) as colourless oil. FTIR (neat, cm^{-1}): 2931 (CH═C), 1450 (C═C), 1116 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.00 (m, 2H, CH_2), 2.92 (t, 2H, J = 7.7 Hz, CH_2), 3.40 (s, 3H, OCH_3), 3.48 (t, 2H, J = 6.4 Hz, OCH_2), 3.97 (s, 3H, OCH_3), 7.37 (d, 1H, J = 8.4 Hz, ArH), 7.47 (m, 1H, ArH), 7.53 (m, 1H, ArH), 7.61 (d, 1H, J = 8.4 Hz, ArH), 7.85 (m, 1H, ArH), 8.12 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.9, 31.3, 59.2, 62.6, 72.9, 122.6, 124.6, 126.0, 126.5, 128.6, 128.9, 128.7, 130.8, 134.4, 154.0; EIMS (*m/z*, %): 230 (M^+, 71), 171 (30), 141 (30), 57 (100). HRMS calcd for $C_{15}H_{18}O_2$ [M+Na] 253.1204, found 253.1201.

4.4. 2-(3-Methoxy-propyl)-naphthalen-1-ol (10)

To a stirred solution of the methyl ether **9** (0.2 g, 0.9 mmol) in dry dichloromethane (20 mL) at 0 °C, boron tribromide (0.3 mL, 3.5 mmol) was added dropwise and the solution was stirred for 1 h at the same temperature. Then water was added and extracted with dichloromethane (3 × 50 mL). The organic phase was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 49:1 v/v hexane–ethyl acetate to afford the desired product **10** (45%) as a brown oil. FTIR (neat, cm^{-1}): 3290 (OH), 1662 (C═C), 1265 (C–O), 1105 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.00 (m, 2H, CH_2), 2.92 (t, 2H, J = 6.4 Hz, CH_2), 3.39 (t, 2H, J = 6.4 Hz, OCH_2), 3.49 (s, 3H, OCH_3), 7.22 (d, 1H, J = 8.3 Hz, ArH), 7.40 (d, 1H, J = 8.3 Hz, ArH), 7.48 (m, 2H, ArH), 7.79 (m, 1H, ArH), 7.94 (s, 1H, OH), 8.33 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.0, 29.9, 59.1, 70.4, 120.5, 123.0, 125.6, 126.2, 127.9, 129.3, 120.0, 125.9, 134.2, 151.1; EIMS (*m/z*, %): 216 (M^+, 63), 184 (100), 156 (59), 128 (50). HRMS calcd for $C_{14}H_{16}O_2$ [M+Na] 239.1048, found 239.1046.

4.5. 3-(1-Benzyloxy-naphthalen-2-yl)-2,2-dimethyl-propan-1-ol (11)

A mixture of naphthol **2** (0.5 g, 2 mmol), potassium carbonate (0.6 g, 4 mmol) and benzyl chloride (0.5 mL, 4 mmol) in acetone (10 mL) was stirred under refluxing for 5 h. After that the reaction mixture was cooled to room temperature. Water was added to the reaction mixture and then extracted with diethyl ether (3 × 50 mL). The combined organic phase was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 23:2 v/v hexane–ethyl acetate to afford the product **11** (95%) as colourless oil. FTIR (neat, cm^{-1}): 3447 (OH), 1467 (C═C), 1077 (C–O), 1044 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.00 (s, 6H, 2 × CH_3), 2.78 (s, 2H, CH_2), 3.05 (d, 2H, J = 7.0 Hz, OCH_2), 3.41 (t, 1H, J = 7.0 Hz, OH), 5.08 (s, 2H, OCH_2), 7.34 (d, 1H, J = 8.4 Hz, ArH), 7.43–7.62 (m, 7H, ArH), 7.65 (d, 1H, J = 8.4 Hz, ArH), 7.91 (m, 1H, ArH), 8.18 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.7 (2 × C), 38.2, 39.0, 69.9, 77.5, 122.6, 124.6, 126.4, 126.8, 128.7, 128.8 (2 × C), 129.3, 129.5 (2 × C), 131.1, 128.2, 128.4, 134.7, 137.0, 153.0; EIMS (*m/z*, %): 320 (M^+, 4), 212 (5), 157 (16), 128 (11), 91 (100). HRMS calcd for $C_{22}H_{24}O_2$ [M+H] 321.1849, found 321.1851.

4.6. 1-Benzyloxy-2-(3-methoxy-2,2-dimethyl-propyl)-naphthalene (12)

To a stirred suspension of sodium hydride (0.12 g, 5 mmol) in dry THF (10 mL), a solution of compound **11** (0.26 g, 0.8 mmol) in dry THF (5 mL) was added dropwise. Then methyl iodide (0.3 mL, 5 mmol) was added dropwise to the reaction mixture. After refluxing for 5 h, the reaction mixture was cooled to room temperature and quenched with water, then extracted with diethyl ether (3 × 30 mL). The combined organic layer was washed with brine and water, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 9:1 v/v hexane–dichloromethane to afford the product **12** (94%) as colourless oil. FTIR (neat, cm^{-1}): 1457 (C═C), 1183 (C–O), 1109 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.00 (s, 6H, 2 × CH_3), 2.85 (s, 2H, CH_2), 3.11(s, 2H, OCH_2), 3.41 (s, 3H, OCH_3), 5.05 (s, 2H, OCH_2), 7.38 (d, 1H, J = 8.4 Hz, ArH), 7.45 (m, 1H, ArH), 7.51 (m, 4H, ArH), 7.64 (m, 3H, ArH), 7.90 (m, 1H, ArH), 8.17(m, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.5 (2 × C), 37.7, 39.3, 59.7, 76.4, 82.1, 122.9, 123.9, 126.1, 126.4, 128.2 (2 × C), 128.5 (2 × C), 129.2 (2 × C), 131.2, 128.8 (2 × C), 134.6, 138.4, 153.8; EIMS (*m/z*, %): 334 (M^+, 4), 212 (22), 157 (20), 128 (13), 91 (100). HRMS calcd for $C_{23}H_{26}O_2$ [M+H] 335.2006, found 335.2006.

4.7. 2-(3-Methoxy-2,2-dimethyl-propyl)-naphthalen-1-ol (13)

To a mixture of compound **12** (0.12 g, 0.37 mmol), potassium iodide (0.18 g, 1.1 mmol) in acetonitrile (6 mL), trimethylsilylchloride (0.14 mL, 1.1 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 1 h. After that water was added to the reaction mixture and extracted with diethyl ether (3 × 30 mL). The combined organic layer was washed with saturated sodium hydrogen carbonate and water, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 19:1 v/v hexane–dichloromethane to afford the desired product **13** (87%) as a colourless solid. Mp 67–68 °C. FTIR (KBr, cm^{-1}): 3229 (OH), 1569 (C═C), 1290 (C–O), 1083 (C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.10 (s, 6H, 2 × CH_3), 2.78 (s, 2H, CH_2), 3.00 (s, 2H, OCH_2), 3.50 (s, 3H, OCH_3), 7.18 (d, 1H, J = 8.3 Hz, ArH), 7.35 (d, 1H, J = 8.3 Hz, ArH), 7.48 (m, 2H, ArH), 7.79 (m, 1H, ArH), 8.36 (m, 1H, ArH), 8.55 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.3 (2 × C), 36.8, 39.5, 59.8, 79.9, 119.3, 123.2, 125.4, 126.2, 127.8, 131.4,

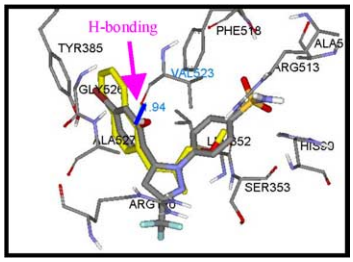


Figure 9. A possible orientation of naphthol **13** in the binding site of COX-2. The hydrogen-bonding interaction is shown as a blue line.

117.9, 125.9, 134.3, 151.7; EIMS (*m*/*z*, %): 244 (M^+, 35), 212 (35), 157 (100), 128 (78) 87 (46). HRMS calcd for $C_{16}H_{20}O_2$ [M+Na] 267.1361, found 267.1359 (Fig. 9).

4.8. Anti-COX (PGHS) assay

4.8.1. Materials. All tissue culture components were purchased from Gibco BRL (Gaithersburg, MD). Aspirin and calcium ionophore A23187 were purchased from Sigma (St. Louis, MO). ^{3}H-PGE_2 was from NEN Life Science (Boston, MA) and anti-PGE_2 antibody was from the Upstate Biotechnology (Upstate, NY) or Sigma (St. Louis, MO).

4.8.2. Cell culture and treatment. Immortalized mouse PGHS-1 (PGHS-$1^{-/-}$) and PGHS-2 (PGHS-$2^{-/-}$) null cells were seeded at 1×10^5 cells/mL in complete Dubelcco's Modified Eagle Medium (DMEM) supplemented with non essential amino acids (0.1 mM), glutamine (292 mg/L), ascorbic acid (50 mg/L) and 10% FCS in 96-well (83 μL/well) flat-bottomed tissue culture plates. The cells were incubated at 37 °C in a humidified incubator with 5% CO_2 for 72 h. The cells were then washed with DMEM medium without FCS and preincubated for 30 min with 83 μL serum-free DMEM medium containing vehicle or drug. Aspirin or the test compounds were dissolved and serially diluted in ethanol or DMSO before they were added to the medium. The final concentrations of ethanol and DMSO were 1% and 0.1%, respectively. Following the preincubation period, the medium was removed and the cells were immediately treated with serum-free medium containing vehicle or drug and 2 μM A23187 for 30 min. Medium samples were then collected from the wells and analyzed for PGE_2 concentration by RIA as previously described. The test compounds were first screened at 10^{-5} g/mL. IC_{50} values were further determined for samples (10^{-5} g/mL) that show inhibitory effect on PGE_2 production. Aspirin, which was found to have PGHS-1 and PGHS-2 IC_{50} values of 0.014 ± 0.008 and 0.058 ± 0.053 mg/mL, respectively was employed as a nonselective COX-2 inhibitor.

4.9. Cytotoxicity assay

The cytotoxicity assay against Vero cells (ATCC CCL-81) was performed employing the colorimetric method.[15] IC_{50} values of the standard compound, ellipticine was found to be 0.4 ± 0.1 μg/mL.

4.10. Molecular modeling

4.10.1. Molecular docking. Crystal structures (1eqh and $1c \times 2$) from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/) provided enzyme structures and binding site information for COX-2 complexed with SC-558[18] and COX-1 bound to flurbiprofen[19,20] as reference structures. Initial structures of eight naphthol compounds (compounds **2**–**13** in Table 1) were generated by molecular modeling software Sybyl 6.8.[21] The geometries of these compounds were subsequently optimized using the semi-empirical parameters AM1.

Binding conformations of naphthol derivatives with COX-2 and COX-1 were analyzed by the program AutoDock 3.0.5[22,23] using the Lamarckian genetic algorithm (LGA)[23] in conjunction with an empirical force field to calculate ligand free energy of binding. Kollman-all-atom[24] charges were assigned to enzyme electrostatic contributions whereas Gasteiger-Hückel[25] charges were assigned to all ligands. All calculations were performed by representing the enzyme affinity by a $90 \times 90 \times 90$ grid box of 0.25 Å grid spacing.

The conditions applied throughout the docking simulations were those, which reproduced the co-crystals of flurbiprofen and SC-558 bound to COX-1 and COX-2 enzymes with root mean square deviation (RMSD) value of 0.7 Å for both cases using flurbiprofen and SC-558 at the binding sites in 1eqh and 1cx2 as references (Fig. 3). The estimated free energy of binding using these docking conditions for SC-558 binding to COX-2 was −11.65 kcal/mol, which was slightly different from the value from the literature (−11.35 kcal/mol). Therefore, the AutoDock method and the parameter set could be extended to search the enzyme binding conformations for other inhibitors accordingly.

Finally, the docked complexes of inhibitor–enzyme were selected according to the criteria of interacting energy combined with geometrical matching quality. All amino acid residues within a 5.0 Å radius of the inhibitor atom were considered and analyzed for their activity contributions.

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