Full Paper

Synthesis and Evaluation of 2-(2-Arylmorpholino)ethyl Esters of Ibuprofen Hydrochlorides as COX-2 and Serotonin Reuptake Inhibitors

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Based on the positive effects of COX-2 inhibitors on depressive symptoms and the desirable physicochemical and biological properties of the morpholine group, a series of novel 2-(2-arylmorpholino)ethyl esters of ibuprofen hydrochlorides were designed, synthesized, and tested for their COX-2 inhibitory and serotonin reuptake inhibitory activities *in vitro*. The structure–activity relationships of the 2-(2-arylmorpholino)ethyl esters of ibuprofen hydrochlorides as dual COX-2 and serotonin reuptake inhibitors were determined and discussed in detail. The biological assays indicated that five of the compounds possess good COX-2 selectivity (selectivity index COX-1/COX-2 42.8–158.1). The compound 2-[2-(4-benzyloxyphenyl)morpholino]ethyl 2-(4-iso-butylphenyl)-propanoate hydrochloride (**1k**) shows better COX-2 inhibitory activity ($IC_{50} = 0.78\ \mu M$) than ibuprofen ($IC_{50} = 7.6\ \mu M$), and it simultaneously possesses favorable serotonin reuptake inhibitory activity.

Keywords: 2-(2-Arylmorpholino)ethyl ester hydrochloride / COX-2 inhibitors / Ibuprofen / Serotonin reuptake inhibitors / Synthesis

Received: July 25, 2013; Revised: September 6, 2013; Accepted: September 11, 2013

DOI 10.1002/ardp.201300279

Introduction

Accumulating evidence demonstrates that depression is associated with increased inflammatory drive, and provoking an acute inflammatory response in healthy humans can result in depression-like behaviors [1–4]. Depression and inflammation are also common among cancer patients. Systemic inflammation may result directly from the tumor, which can release proinflammatory cytokines, or it may arise as cancer cells, which are identified as a kind of foreign body by the immune system [2, 5]. A plausible biological pathway linking inflammation and depression is the activation of indoleamine 2,3-dioxygenase [6]. In addition, impaired glucocorticoid receptor (GR) function has been characterized by increased circulation and expression of proinflammatory cytokines, which have been found to negatively regulate GR function through their signaling pathways.

Celecoxib, a classic COX-2 inhibitor, has also been demonstrated to significantly increase nuclear localization of the GR, which expands its potential clinical application to multiple disorders associated with impaired GR function including major depression [7]. Moreover, the inhibition of COX-2 was reported to limit the impact of stressors. Treatment with celecoxib prevented the deregulation of the hypothalamic pituitary adrenal (HPA) axis, in particular with regard to the increase of cortisol, one of the key biological features associated with depression, while treatment with a COX-1 inhibitor did not prevent HPA-axis dysregulation [7, 8]. In addition, in a randomized, double-blind trial in patients with major depression, celecoxib plus fluoxetine resulted in a statistically significant better outcome related to improvements in major depression symptoms compared with fluoxetine-alone monotherapy [9]. Accordingly, a clinical antidepressant effect of rofecoxib was observed in patients with osteoarthritis among which 15% had a comorbid

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depressive syndrome, as evaluated by a specific depression self-report, a significant decrease (from 15% to 3% of patients) in the rate of substantive depression during therapy with rofecoxib [10]. Taken together, it is not difficult to predict that the potential COX-2 inhibitors that possess both considerable anti-inflammatory activity and possible antidepressant activity will be widely used in the treatment of multiple disorders associated with inflammation and depression and even in the auxiliary treatment of cancer.

Morpholine analogs have shown good anti-inflammatory and antioxidant activity. Kourounakis and co-workers [11] synthesized a variety of 2,6-di-*tert*-butylphenol, which are amides or amines of morpholine residues, some of which exhibited anti-inflammatory and antioxidant activities. Naproxen [12] and indomethacin [13] as traditional non-steroidal anti-inflammatory drugs (NSAIDs) were modified with 2-(2-arylmorpholino)ethanols, which have shown better COX-2 inhibitory activities *in vitro* and lower gastrointestinal irritation side effects by occupying the additional side pocket of the COX-2 enzyme and the antioxidant activity of the morpholine moiety.

Morpholine ring possessing specially chemical and physicochemical properties was widely utilized to modify biological properties of various drugs. Reboxetine [14], moclobemide [15], and viloxazine [16], known as good antidepressants, all share a morpholine scaffold in their structure (Fig. 1). It has been demonstrated that reboxetine, as an α-aryloxybenzyl derivative of morpholine, is metabolized via oxidation of the morpholine ring, *O*-dealkylation of the ethoxyphenoxy ring, and hydroxylation. Some evidences also suggest that, compared with fluoxetine, reboxetine may be more effective in severely depressed patients in improving social behavior. Limited long-term data suggest that reboxetine may prevent relapse in patients who respond to short-term therapy [17, 18]. Other novel morpholine derivatives with potent antidepressant effects were also reported recently [19–22]. One study [23] reported the pharmacological and biochemical properties of a new compound, 2-(7-indeyloxymethyl)morpholine hydrochloride, which has been confirmed possessing less effect on the uptake of either serotonin (5-HT) or norepinephrine (NE). This compound has a novel profile as an antidepressant agent, which is quite different from that of either viloxazine or tricyclic compounds. Two US patents [24, 25] also reported the structure and synthesis of a series of morpholine derivatives and provided a pharmaceutical composition. This research makes it possible to use morpholine derivatives as antidepressant drugs.

Based on the positive effects of COX-2 inhibitors on depressive symptoms and on the desirable physicochemical and biological properties of the morpholinyl group, the free carboxylic moiety of a NSAID ibuprofen, which is restricted due to its gastrointestinal irritation side effects, after long-term use [26] was modified with 2-(2-arylmorpholino)ethyl esters to increase its COX-2 inhibitory activity based on the structural differences between COX-1 and COX-2 [27–30]. We suppose that these novel COX-2 inhibitors could exhibit good stability and show both favorable COX-2 inhibitory activity and antidepressant property (Fig. 2). Then, a series of compounds were obtained and tested *in vitro* for the evaluation of their COX-2 inhibitory and antidepressant activities. And according to the biological assay, these derivatives of ibuprofen show both good COX-2 inhibitory activity and antidepressant property. The liposolubility and bioavailability of these compounds in *in vivo* tests will be the subject of a separate report soon.

The target molecules 2-(2-arylmorpholino)ethyl ester of ibuprofen hydrochlorides (**1a**–**1l**) were synthesized using the sequence of reactions illustrated in Scheme 1.

Results and discussion

Synthesis

Ibuprofen was converted into 2-(4-iso-butylphenyl)propanoyl chloride (**3**) through the reaction with thionyl chloride. And 2-(2-phenylmorpholino)ethanol (**2a**) formed via the Leuckart–Wallach reaction [31] without purification was reacted with the compound (**3**) in the presence of THF and Et_3N. Then the obtained ester as an oily product was converted into its hydrochloride derivative (**1a**) as a white solid. Other products (**1b**–**1l**) were obtained in the similar way by reacting their respective esters with hydrogen chloride. We found that the substituent Ar has great influence on the cyclization reaction. The compounds (**1g**–**1l**) substituted with naphthyl or benzyloxyphenyl have a higher yield than those substituted with phenyl; the order of yield is 6-MeO-2-$C_{10}H_6$ > 4-$PhCH_2OC_6H_4$ > C_6H_5. The synthesis method was high-yield, safe, operationally simple, and cost-effective, meeting with the requirements of contemporary organic synthesis, and was

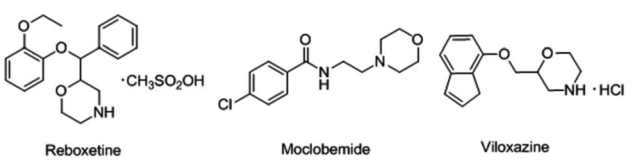


Figure 1. Morpholine scaffold in common antidepressants.

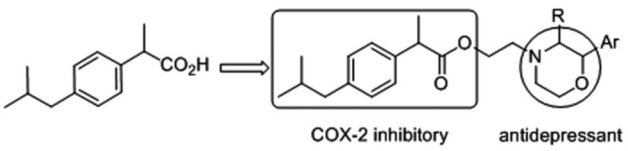
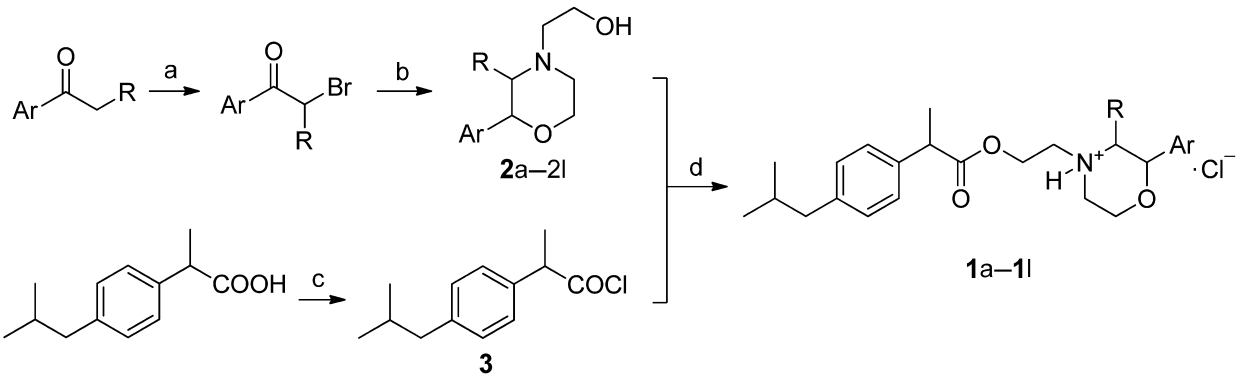


Figure 2. The structure design on morpholine-substituted ibuprofen derivatives expected to be dual-targeted compounds.



a: Ar = C_6H_5, R = H; **b**: Ar = 4-MeC_6H_4, R = H; **c**: Ar = 4-EtC_6H_4, R = H;
d: Ar = 4-MeOC_6H_4, R = H; **e**: Ar = 4-*i*-PrOC_6H_4, R=H; **f**: Ar = 2, 4-Cl_2-5-FC_6H_2, R=H;
g: Ar = 6-MeO-2-$C_{10}H_6$, R = H; **h**: Ar = 5-Cl-6-MeO-2-$C_{10}H_5$, R= H; **i**: Ar = 5-Br-6-MeO-2-$C_{10}H_5$, R = H;
j: Ar = 6-MeO-2-$C_{10}H_6$, R = CH_3; **k**: Ar = 4-Ph$CH_2OC_6H_4$, R = H; **l**: Ar = 4-Ph$CH_2OC_6H_4$, R = CH_3

Scheme 1. Synthesis of the molecules. Reagents and conditions: (a) $CuBr_2$, EtOH, reflux, 6 h; (b) (i) $HN(CH_2CH_2OH)_2$, NMP, 60°C, 2 h; (ii) HCO_2H, 180°C, 18 h; (c) $SOCl_2$, C_6H_6, reflux, 5 h; and (d) (i) THF, Et_3N, rt, 24 h; (ii) HCl (g), Et_2O then $(CH_3)_2CO$.

reported in [13]. All of the compounds were characterized by ^{1}H NMR and elemental analysis.

Biological *in vitro* evaluation

All of the new compounds synthesized were tested *in vitro* initially at 10 μM for selectivity and potency against human COX-1 and COX-2 enzymes. On the basis of their *in vitro* efficacy, selected compounds (**1g–1l**) were tested against COX-1 at 0.1, 1.0, 10.0, and 100 μM, respectively, while against COX-2 at 0.01, 0.1, 1.0, and 10.0 μM, respectively. The IC_{50} values for COX-1 and COX-2 were determined for selected/promising compounds. Celecoxib was used as control group for the *in vitro* COXs inhibitory assay. The concentration of the selected/promising compounds for 50% inhibition of COX-1 and COX-2 (IC_{50}) was calculated by the software SPSS. The results are shown in Table 1.

COX-2 inhibitory activity and selectivity

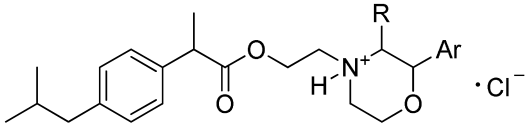
Ibuprofen is a non-selective inhibitor of COX isozymes; the IC_{50} values of COX-2 and COX-1 are 7.6 and 20 μmol/L, respectively [32]. Biological evaluation of the target molecules showed that the conversion of the free acid group of the non-selective inhibitor ibuprofen to 2-(2-arylmorpholino)ethyl esters gives rise to compounds possessing good COX-2 selectivity (selectivity index COX-1/COX-2 42.8–158.1).

According to the analysis of the result of biological activity assay, the possible structure–activity relationship of compounds **1a–1l** is shown in Fig. 3. On the whole, choosing a suitable size of Ar groups, which has been confirmed by the comparison between phenyl-, naphthyl-, and benzyloxyphenyl-substituted derivatives according to **1a–1l**, is a major factor in maintaining potent COX-2 inhibitory activity. Increasing the size of Ar groups appropriately is benefit to improving the selectivity index (COX-1/COX-2), while an oversize group often results in the decrease in COX-1 and COX-2 inhibitory activity, as the IC_{50} shown in Table 1. 6-Methoxy naphthyl substituted morpholine derivative (**1j**) shows the best COX-2 inhibitory activity ($IC_{50} = 0.038$ μM). This may be due to the morpholino-3-methyl, which made compound **1j** suitable for taking the side pocket of COX-2. Plausibly, it may increase the biological activity when CH_3– replaced in R instead of H– according to the comparison of COX-2 inhibitory activity of **1j** and **1g**. In addition, compound **1h** ($IC_{50} = 0.075$ μM) shows better COX-2 inhibitory activity than compound **1i** ($IC_{50} = 0.16$ μM). Replacement of bromine atom with chlorine could increase the selectivity, and it is probably because chlorine atom could form hydrogen bonds with amino acid residues more easily than bromine atom. 4-Benzyloxyphenyl substituted morpholine derivative (**1k**) shows good COX-2 inhibitory and selectivity.

Serotonin reuptake inhibitory activity

The compounds (**1g–1l**) possessing favorable COX-2 inhibitory activity were tested *in vitro* for the inhibition of SERT at the concentration of 10 mg/L. Fluoxetine was used as control group for the *in vitro* SERT inhibitory assay (Table 1). Results revealed that several compounds also displayed substantial serotonin reuptake inhibition. The compound **1h** (inhibition of SERT 30.0%) also shows better SERT inhibitory activity than compound **1i** (inhibition of SERT 5.5%), and it can be supposed that the mechanism of chlorine atom in SERT inhibition is similar to its function in COX-2 inhibition. Moreover, the

Table 1. The COX inhibitory activity and the inhibition rate of SERT of compounds **1a–1l**.



1a–1l

Ar	R	Compd	Selectivity index (COX-1/COX-2)	IC_{50} (μmol/L) COX-2	IC_{50} (μmol/L) COX-1	Inhibition on SERT (%) (at 10 mg/L)
C_6H_5	H	**1a**	–	>100	>100	–
4-MeC_6H_4	H	**1b**	–	>100	>100	–
4-EtC_6H_4	H	**1c**	–	>100	>100	–
4-$MeOC_6H_4$	H	**1d**	–	>100	>100	–
4-*i*-$PrOC_6H_4$	H	**1e**	–	>100	>100	–
2, 4-Cl_2-5-FC_6H_2	H	**1f**	–	>100	>100	–
6-MeO-2-$C_{10}H_6$	H	**1g**	42.8	0.09	3.85	19.4
5-Cl-6-MeO-2-$C_{10}H_5$	H	**1h**	43.4	0.075	1.76	30.0
5-Br-6-MeO-2-$C_{10}H_5$	H	**1i**	158.1	0.16	25.3	5.5
6-MeO-2-$C_{10}H_6$	CH_3	**1j**	46.3	0.038	1.76	12.1
4-$PhCH_2OC_6H_4$	H	**1k**	60.5	0.78	47.2	49.9
4-$PhCH_2OC_6H_4$	CH_3	**1l**	0.88	78.4	69.5	14.6
		Celecoxib	214.0	0.05	10.7	
		Ibuprofen [32]	2.6	7.6	20.0	
		Fluoxetine				55.0

compound **1k** shows good SERT inhibitory activity (inhibition of SERT 49.9%), which is related to its COX-2 inhibitory activity.

Conclusion

With the aim of developing novel compounds possessing both COX-2 and SERT inhibitory activity, we have designed, synthesized, and tested a series of 2-(2-arylmorpholino)ethyl ester of ibuprofen hydrochlorides, and some of them, especially **1h** and **1k**, displayed dual COX-2 and SERT inhibitory activities. The compound **1k** shows good COX-2 selective inhibitory property and its anti-inflammatory activity is higher than that of ibuprofen. At the same time, compound **1k** possesses favorable antidepressant activity compared with fluoxetine. It is identified as the most promising compound among these scaffolds, the further exploration *in vivo* model of which will be reported in future publications. What is more, we have formed the structure–activity relationship of 2-(2-arylmorpholino)ethyl ester of ibuprofen hydrochlorides as dual COX-2 and SERT inhibitors, which will be vital for further development of more effective drugs and a significant reference for similar research.

Experimental

Instruments

All reagents and solvents were of commercial quality and used without further purification. Reactions were performed under the protection of nitrogen and monitored by thin-layer chromatography (TLC) on silica gel plates, visualizing with ultraviolet light or iodine spray. Flash chromatography was

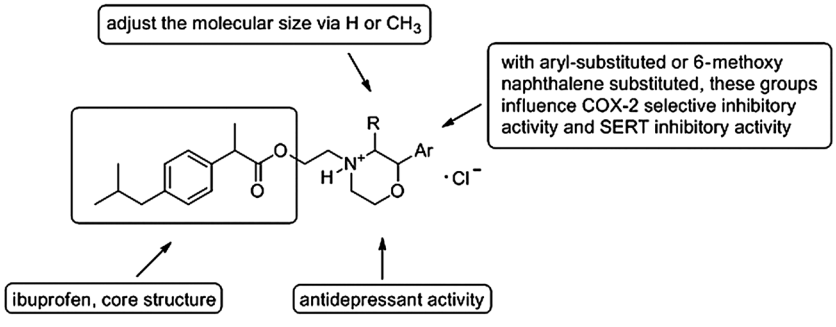


Figure 3. The structure–activity relationship of compounds **1a–11**.

performed on silica gel (200–400 mesh) using commercially available petroleum ether, ethyl acetate. Melting points (m.p.) were taken in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker 400 MHz spectrometer. Chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm, and signals are given as follows. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet) as well as br (broad). Coupling constants (J) are given in Hertz. Microanalysis was performed using a C H N S/O analyzer. Elemental data are within ±0.2%. Sodium thioglycollate (Difco). Calcium cation ionophore A23187, lipopolysaccharides (LPS), dimethyl sulfoxide (DMSO), cecoxib (Sigma), new calf serum (NCS, 5%, Gibco), square-free kit (PGE_2 and 6-keto-$PGF_{1\alpha}$, Institute of Immune Technology of East Asia). 2-(2-Arylmorpholino)ethanols were synthesized following [12].

Synthesis of the title compounds

*Synthesis of 2-(4-iso-butylphenyl)propanoyl chloride (**3**)*

Ibuprofen (1.95 mmol) and thionyl chloride (5.48 mmol) in benzene (5 mL) were refluxed for 5 h, until the evolution of hydrogen chloride and sulfur oxide ceased. The excess thionyl chloride and benzene were distilled off under reduced pressure, to give yellow liquid 2-(4-iso-butylphenyl)propanoyl chloride (**3**).

*General procedure for the synthesis of 2-(2-arylmorpholino)-ethyl ester of ibuprofen hydrochlorides **1a–1l***

2-(4-iso-Butylphenyl)propanoyl chloride (**3**) prepared before was dissolved in 10 mL THF; then the solution of 2-(2-arylmorpholino) ethanols (**2a–2l**, 3.9 mmol) as well as triethylamine (1 mL) was slowly added. The reaction mixture was stirred at room temperature for 24 h and filtered to remove triethylamine hydrochloride. The filtrate was distilled under reduced pressure to remove tetrahydrofuran; then the residue was dissolved in ethyl acetate and washed in sodium carbonate (6%), dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The free base was obtained as oily product by flash chromatography, eluting with petroleum ether/ethyl acetate. Then the oily product was dissolved in proper ether and treated with ethereal hydrogen chloride, followed by filtration, and the residue was washed with acetone to give hydrochlorides.

*2-(2-Phenylmorpholino)ethyl 2-(4-iso-butylphenyl)-propanoate hydrochloride (**1a**)*

Yellow solid, m.p. 119–122°C, yield 54.1%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.81–0.91 (m, 6H, $2 \times CH_3$), 1.48 (dd, $J = 2.8$ Hz, $J = 7.2$ Hz, 3H, CH_3), 1.83 (m, 1H, CH), 2.22–2.28 (m, 2H, C_4H_7NO 5-H), 2.42 (m, 2H, CH_2), 3.00–3.24 (bm, 4H, NCH_2, C_4H_7NO 3-H), 3.70 (m, 1H, CH), 3.90 (tt, $J = 8.8$ Hz, $J = 2.8$ Hz, 1H, C_4H_7NO 6-H*a*), 4.12 (bs, 1H, C_4H_7NO 6-H*e*), 4.53 (bs, 2H, OCH_2), 4.94 (br, 1H, C_4H_7NO 2-H), 7.10–7.70 (m, 9H, C_6H_4, C_6H_5). Elemental analysis found C, 69.48; H, 7.91; N, 3.25; $C_{25}H_{34}O_3NCl$ requires C, 69.51; H, 7.93; N, 3.24.

*2-[2-(p-Tolyl)morpholino]ethyl 2-(4-iso-butylphenyl)-propanoate hydrochloride (**1b**)*

Yellow oil, yield 56.1%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.89 (dd, $J = 0.8$ Hz, $J = 6.8$ Hz, 6H, $2 \times CH_3$), 1.48 (d, $J = 6.8$ Hz, 3H, CH_3), 1.83 (m, 1H, CH), 2.05–2.24 (m, 2H, C_4H_7NO 5-H), 2.34 (s, 3H, CH_3), 2.43 (dd, $J = 2.0$ Hz, $J = 6.8$ Hz, 2H, CH_2), 2.59–2.69 (m, 3H, NCH_2, C_4H_7NO 3-H*a*), 2.84–2.87 (m, 1H, C_4H_7NO 3-H*e*), 3.67–3.75 (m, 2H, CH, C_4H_7NO 6-H*a*), 3.90–3.94 (m, 1H, C_4H_7NO 6-H*e*), 4.21–4.24 (bm, 2H, OCH_2), 4.44–4.246 (bm, 1H, C_4H_7NO 2-H), 7.04–7.26 (m, 8H, C_6H_4, C_6H_4). Elemental analysis found C, 70.03; H, 8.12; N, 3.11; $C_{26}H_{36}O_3NCl$ requires C, 70.01; H, 8.14; N, 3.14.

*2-[2-(4-Ethylphenyl)morpholino]ethyl 2-(4-iso-butylphenyl)-propanoate hydrochloride (**1c**)*

White solid, m.p. 134–136°C, yield 35.1%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.89 (dd, $J = 0.8$ Hz, $J = 6.8$ Hz, 6H, $2 \times CH_3$), 1.22 (t, $J = 8.0$ Hz, 3H, CH_3), 1.48 (m, 3H, CH_3), 1.83 (m, 1H, CH), 2.22–2.28 (m, 2H, C_4H_7NO 5-H), 2.43 (dd, $J = 1.2$ Hz, $J = 7.2$ Hz, 2H, CH_2), 2.60–2.69 (m, 5H, CH_2, NCH_2, C_4H_7NO 3-H*a*), 2.87–2.90 (m, 1H C_4H_7NO 3-H*e*), 3.68–3.75 (m, 2H, CH, C_4H_7NO 6-H*a*), 3.91 (bm, 1H, C_4H_7NO 6-H*e*), 4.23–4.26 (bm, 2H, OCH_2), 4.48 (bm, 1H, C_4H_7NO 2-H), 7.03–7.26 (m, 8H, C_6H_4, C_6H_4). Elemental analysis found C, 70.54; H, 8.28; N, 3.05; $C_{27}H_{38}O_3NCl$ requires C, 70.49; H, 8.33; N, 3.04.

*2-[2-(4-Methoxyphenyl)morpholino]ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1d**)*

Yellow oil, yield 40.6%, ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.89 (dd, $J = 0.8$ Hz, $J = 6.8$ Hz, 6H, $2 \times CH_3$), 1.48 (d, $J = 6.8$ Hz, 3H, CH_3), 1.83 (m, 1H, CH), 2.04–2.26 (m, 2H, C_4H_7NO 5-H), 2.43 (d, $J = 6.8$ Hz, 2H, CH_2), 2.57–2.68 (m, 3H, NCH_2, C_4H_7NO 3-H*a*), 2.81–2.84 (m, 1H, C_4H_7NO 3-H*e*), 3.67–3.74 (m, 2H, CH, C_4H_7NO 6-H*a*), 3.82 (s, 3H, OCH_3), 3.89–3.92 (m, 1H, C_4H_7NO 6-H*e*), 4.17–4.26 (m, 2H, OCH_2), 4.41 (bm, 1H, C_4H_7NO 2-H), 6.85–7.26 (m, 8H, C_6H_4, C_6H_4). Elemental analysis found C, 67.58; H, 7.81; N, 3.05; $C_{26}H_{36}O_4NCl$ requires C, 67.59; H, 7.85; N, 3.03.

*2-[2-(4-iso-Propoxyphenyl)morpholino]ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1e**)*

Yellow oil, yield 33.9%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.89 (dd, $J = 0.8$ Hz, $J = 6.8$ Hz, 6H, $2 \times CH_3$), 1.32 (d, $J = 6.4$ Hz, 6H, $2 \times CH_3$), 1.48 (d, $J = 6.8$ Hz, 3H, CH_3), 1.82 (m, 1H, CH), 2.10–2.28 (m, 1H, C_4H_7NO 5-H*a*), 2.43 (d, $J = 6.8$ Hz, 2H, CH_2), 2.68–2.72 (m, 3H, NCH_2, C_4H_7NO 3-H*a*), 3.12 (m, 1H, C_4H_7NO 5-H*e*), 3.42–3.54 (bm, 1H, C_4H_7NO 3-H*e*), 3.68–3.87 (m, 3H, C_4H_7NO 6-H, CH), 4.19–4.24 (m, 2H, OCH_2), 4.38–4.42 (m, 1H, C_4H_7NO 2-H), 4.54 (m, $J = 6.4$ Hz, 1H, CH), 6.80–7.26 (m, 8H, C_6H_4, C_6H_4). Elemental analysis found C, 68.58; H, 8.21; N, 2.85; $C_{28}H_{40}O_4NCl$ requires C, 68.62; H, 8.23; N, 2.86.

*2-[2-(2,4-Dichloro-5-fluorophenyl)morpholino]ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1f**)*

White solid, m.p. 185–188°C, yield 63.5%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.88–0.93 (m, 6H, $2 \times CH_3$), 1.48 (dd $J = 7.6$ Hz, $J = 5.6$ Hz, 3H, CH_3), 1.83 (m, 1H, CH), 2.18–2.29 (bs, 1H, C_4H_7NO 5-H*a*), 2.37–2.45 (m, 2H, CH_2), 2.52–2.61 (bs, 1H, C_4H_7NO 5-H*e*), 2.98–3.10 (bm, 1H, C_4H_7NO 3-H*a*), 3.17–3.24 (m, 2H, NCH_2), 3.57 (d, 1H, $J = 9.2$ Hz, C_4H_7NO 3-H*e*), 3.67–3.73 (m, 1H, CH), 3.85–3.97 (m, 1H, C_4H_7NO 6-H*a*), 4.37–4.46 (m, 1H, C_4H_7NO 6-H*e*), 4.70 (t, $J = 7.2$ Hz, 2H, OCH_2), 5.47 (t, 1H, $J = 7.2$ Hz, C_4H_7NO 2-H), 6.92–7.49 (m, 6H, C_6H_4, C_6H_2), 14.05 (bs, 1H, HCl). Elemental analysis found C, 57.88; H, 6.01; N, 2.72; $C_{25}H_{31}O_3NFCl_3$ requires C, 57.87; H, 6.02; N, 2.70.

2-[2-(6-Methoxynaphthalen-2-yl)morpholino]ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1g**)

White solid, m.p. 168–170°C, yield 45.0%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.84–0.90 (m, 6H, 2 × CH_3), 1.47 (m, 3H, CH_3), 1.71–1.80 (m, 1H, CH), 2.31–2.38 (m, 2H, CH_2), 2.44 (bs, 1H, C_4H_7NO 5-H*a*), 2.62 (bs, 1H, C_4H_7NO 5-H*e*), 3.16–3.19 (bm, 1H, C_4H_7NO 3-H*a*), 3.19–3.25 (m, 2H, NCH_2), 3.43–3.55 (dd, 1H, J = 0.8 Hz, J = 4.0 Hz, C_4H_7NO 3-H*e*), 3.66–3.71 (br, 1H, CH), 3.97–3.99 (m, 1H, C_4H_7NO 6-H*a*), 3.93 (s, 3H, OCH_3), 4.38–4.41 (m, 1H, C_4H_7NO 6-H*e*), 4.30–4.74 (m, 2H, OCH_2), 5.32 (t, 1H, J = 7.2 Hz, C_4H_7NO 2-H), 6.84–7.78 (m, 10H, C_6H_4, $C_{10}H_6$). Elemental analysis found C, 70.37; H, 7.51; N, 2.75; $C_{30}H_{38}O_4NCl$ requires C, 70.36; H, 7.48; N, 2.74.

2-[2-(5-Chloro-6-methoxynaphthalen-2-yl)morpholino]-ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1h**)

Pale white solid, m.p. 119–122°C, yield 59.5%, ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.77–0.86 (m, 6H, 2 × CH_3), 1.33–1.38 (d, J = 7.6 Hz, 3H, CH_3), 1.80 (m, 1H, CH), 2.09–2.22 (m, 2H, CH_2), 3.20–3.25 (bm, 3H, NCH_2, C_4H_7NO 5-H*a*), 3.57–3.64 (m, 2H, C_4H_7NO 5-H*e*, CH), 3.74–3.83 (m, 2H, C_4H_7NO 3-H), 4.01 (s, 3H, OCH_3), 4.05–4.12 (m, 2H, OCH_2), 4.20–4.24 (m, 1H, C_4H_7NO 6-H*a*), 4.46 (bs, 1H, C_4H_7NO 6-H*e*), 5.07 (m, 1H, C_4H_7NO 2-H), 6.84–8.13 (m, 9H, C_6H_4, $C_{10}H_5$), 11.56 (bs, 1H, HCl). Elemental analysis found C, 65.89; H, 6.81; N, 2.54; $C_{30}H_{37}O_4NCl_2$ requires C, 65.93; H, 6.82; N, 2.56.

2-[2-(5-Bromo-6-methoxynaphthalen-2-yl)morpholino]-ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1i**)

Brown solid, m.p. 168–171°C, yield 71.2%, ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.84–0.90 (m, 6H, 2 × CH_3), 1.47 (d, J = 7.6 Hz, 3H, CH_3), 1.75 (m, 1H, CH), 2.28–2.38 (m, 2H, CH_2), 2.62 (bs, 1H, C_4H_7NO 5-H*a*), 3.08–3.25 (bm, 3H, NCH_2, C_4H_7NO 5-H*e*), 3.45–3.55 (br, 1H, C_4H_7NO 3-H*a*), 3.62–3.80 (m, 2H, C_4H_7NO 3-H*e*, CH), 3.93–4.01 (m, 1H, C_4H_7NO 6-H*a*), 4.05 (s, 3H, OCH_3), 4.40 (m, 1H, C_4H_7NO 6-H*e*), 4.70 (m, 2H, OCH_2), 5.37 (br, 1H, C_4H_7NO 2-H), 6.82–8.26 (m, 9H, C_6H_4, $C_{10}H_5$), 13.63 (bs, 1H, HCl). Elemental analysis found C, 60.95; H, 6.41; N, 2.33; $C_{30}H_{37}O_4NClBr$ requires C, 60.97; H, 6.37; N, 2.31.

2-[2-(6-Methoxynaphthalen-2-yl)-3-methylmorpholino]-ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1j**)

Pale yellow solid, m.p. 184–187°C, yield 87.6%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.72 (dd, J = 6.0 Hz, J = 8.8 Hz, 3H, CH_3), 0.84–0.87 (m, 6H, 2 × CH_3), 1.47 (d, J = 7.6 Hz, 3H, CH_3), 1.79 (m, 1H, CH), 2.36–2.42 (m, 2H, CH_2), 2.54–2.62 (m, 2H, NCH_2), 2.68–2.72 (m, 1H, C_4H_6NO 5-H*a*), 2.91–3.02 (m, 1H, C_4H_6NO 5-H*e*), 3.65–3.75 (m, 2H, CH, C_4H_6NO 3-H), 3.81 (s, 5H, OCH_2, OCH_3), 4.14 (m, 1H, C_4H_6NO 6-H*a*), 4.21 (m, 1H, C_4H_6NO 6-H*e*), 7.03–7.68 (m, 10H, C_6H_4, $C_{10}H_6$). Elemental analysis found C, 70.79; H, 7.61; N, 2.65; $C_{31}H_{40}O_4NCl$ requires C, 70.77; H, 7.66; N, 2.66.

2-[2-(4-Benzyloxyphenyl)morpholino]ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1k**)

Pale yellow solid, m.p. 147–149°C, yield 61.2%, ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.88–0.91 (m, 6H, 2 × CH_3), 1.48 (m, 3H, CH_3), 1.83 (m, 1H, CH), 2.42 (d, 2H, J = 7.6 Hz, CH_2), 2.23 (m, 1H, C_4H_7NO 5-H*a*), 3.00–3.23 (m, 3H, NCH_2, C_4H_7NO 3-H*a*), 2.54 (m, 1H, C_4H_7NO 5-H*e*), 3.37 (m, 1H, C_4H_7NO 3-H*e*), 3.70 (m, 1H, CH), 3.86 (m, 1H, C_4H_7NO 6-H*a*), 4.20 (m, 1H, C_4H_7NO 6-H*e*), 4.68 (bs, 2H, OCH_2), 5.08 (d, 2H, $PhCH_2O$), 5.10–5.13 (m, 1H, C_4H_7NO 2-H), 6.96–7.42 (m, 13H, 2 × C_6H_4, C_6H_5), 13.59 (bs, 1H, HCl). Elemental analysis found C, 71.40; H, 7.51; N, 2.63; $C_{32}H_{40}O_4NCl$ requires C, 71.42; H, 7.49; N, 2.60.

2-[2-(4-Benzyloxyphenyl)-3-methylmorpholino]ethyl 2-(4-iso-butylphenyl)propanoate hydrochloride (**1l**)

Yellow oil, yield 54.5%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 0.76 (m, 3H, CH_3), 0.88–0.90 (m, 6H, 2 × CH_3), 1.49 (m, 3H, CH_3), 1.82 (m, 1H, CH), 2.42 (m, 2H, CH_2), 2.52–2.61 (m, 2H, NCH_2), 2.72 (m, 1H, C_4H_6NO 5-H*a*), 3.01 (m, 1H, C_4H_6NO 5-H*e*), 3.65–3.72 (m, 3H, OCH_2, CH), 3.81 (m, 1H, C_4H_6NO 3-H), 3.95 (m, 1H, C_4H_6NO 2-H), 4.12–4.31 (m, 2H, C_4H_6NO 6-H), 5.06 (d, 2H, $PhCH_2O$), 6.92–7.41 (m, 13H, 2 × C_6H_4, C_6H_5). Elemental analysis found C, 71.76; H, 7.64; N, 2.55; $C_{33}H_{42}O_4NCl$ requires C, 71.78; H, 7.67; N, 2.54.

Biological assay

COX-1/2 inhibitory assay

The methods that followed [13] were shown as follows.

Peritoneal macrophages were obtained from mice that were euthanized by cervical dislocation. The peritonea of the animals were surgically exposed using a midline incision. An experimental group of mice were administered an intraperitoneal injection of 2 mL of 3% m/v sodium thioglycollate four days prior to the sacrifice. Following the removal of the carotid artery, the excised tissue was immersed in 75% ethanol for 1–2 min; the peritoneal fluid was then harvested by injecting D-Hanks into the peritoneal cavity and subsequent syringe aspiration. Cell suspensions were pelleted by centrifugation and washed with D-Hanks two times.

Cells were incubated in PRMI 1640 complete medium supplemented with 5% NCS for 4 h at 37°C with 5% CO_2 in a humidified chamber.

The mouse peritoneal macrophages were seeded in 48-well plates (1 × 109/L/well), cultured for 2 h. Cultures were removed and washed twice with D-Hanks. PRMI 1640 complete medium supplemented with 5% NCS was added into each well, then processed according to the following groups: (i) negative control group of DMSO (control); (ii) A23187 (final concentration 1 μmol/L); (iii) A23187 + **10a–10g** (5 μmol/L); (iv) A23187 + celecoxib (1 μmol/L). Experiments were performed at least three times in triplicate. The peritoneal macrophages, drugs, or solvents were incubated for 1 h at 37°C with 5% CO_2; then A23187 was added (final concentration 1 μmol/L) and incubated for 1 h under the same conditions. The supernatant was collected and the concentration of 6-keto-$PGF_{1\alpha}$ was tested with square-free kit marked with ^{125}I. The standard curve was made following the method supplied, and the concentration of the tested samples was calculated according to the measured values, respectively.

The COX-2 inhibitory activity was processed according to the following four groups: (i) negative control group of DMSO (control); (ii) LPS (final concentration 1 mg/L); (iii) LPS + **10a–10g** (5 μmol/L); (iv) LPS + celecoxib (1 μmol/L). The peritoneal macrophages, drugs, or solvents were also incubated for 1 h at 37°C with 5% CO_2; then LPS was added (final concentration 1 mg/L) and incubated for 9 h under the same conditions. The supernatant was collected and the concentration of PGE_2 was tested with square-free kit marked with ^{3}H.

Serotonin reuptake inhibitory assay

RBL-2H3 cell line, purchased from China Center for Type Culture Collection (CCTCC), was maintained in 85% MEM with Earle's

salts supplemented with 1.5 g/L $NaHCO_3$, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate, and 2.0 mM L-glutamine with 15% bovine calf serum at 37°C. The cells were washed with D-Hanks twice until they achieved logarithmic phase in good condition; then 0.125% trypsin–EDTA solution was added and placed for 5 min at 37°C. Cell suspensions were pelleted by centrifugation and purified liquid was removed; then cells were incubated in PRMI 1640 complete medium and resuspended at 6×10^5 per milliliter.

CACO-2 cell line, purchased from National Center for Pharmaceutical Screening (NCPC), was maintained under the same conditions just as RBL-2H3 cells did.

RBL-2H3 cells (4×10^5) were seeded into 96-well plates at 100 μL per plate. The absorbance was measured at 475 nm (excitation wavelength) and 605 nm (emission wavelength) after the cells proliferated for 10 h.

CACO-2 cells (1×10^5) were seeded into 96-well plates at 100 μL per plate. The absorbance was measured at 475 nm (excitation wavelength) and 605 nm (emission wavelength) after the cells proliferated for 6–7 days.

The inhibition ratio was calculated as follows: (cpm of sample – cpm of background)/(cpm of maximum – cpm of background).

This work was supported by the key project of Natural Science Foundation of Hunan province (Grant No.07JJ3022).

The authors have declared no conflict of interest.

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