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Design, Synthesis and Biological Evaluation of 2-(2-Aryl-morpholino-4-yl)ethyl Esters of Indomethacin as Potential Cyclooxygenase-2 (COX-2) Inhibitors†

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A number of novel 2-(2-arylmorpholino-4-yl)ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochlorides were synthesized and tested for their cyclooxygenase (COX-1 and COX-2) inhibition properties *in vitro*. Many of these compounds exhibited moderate to good selective COX-2 inhibition, and subtle structural changes in the substituents on the side chain of the ester moiety altered the inhibitory properties significantly. 2-[2-(4-Butoxyphenyl)morpholino-4-yl]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1f**), showed good selective COX-2 inhibitory activity (Selective index (SI) 182), which is comparative with celecoxib (SI 214), a COX-2 inhibitor of diarylpyrazoles. While 2-[2-(2,4-dichloro-5-fluorophenyl)morpholino-4-yl]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1g**), showed greater selective COX-2 inhibitory activity (SI 358) than celecoxib. Both compounds were identified as compromising derivatives in this class to reduce the side effects generated by nonsteroidal anti-inflammatory drugs (NSAIDs) indomethacin.

Keywords indomethacin, 2-(2-arylmorpholino-4-yl)ethanol, synthesis, COX-2 selective inhibition, antioxidant

Introduction

Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) remain the most commonly used medications for the treatment of many inflammatory diseases, however, the toxicity of NSAIDs substantially limit their long term use.[1] The essential fatty acid arachidonic acid (AA) which is released from membrane phospholipids by various stimuli is metabolized into inflammatory factors protaglandins (PGs), thromboxane A2 (TXA2) and leukotries (LTs).[2,3] Cyclooxygenases (COXs) and lipoxygenase (LOX) play important roles during the two metabolic routes, thus exploitation of dual-targeted compounds may be a new method in NSAIDs pharmacology.[4-6] The results on lipid peroxidation largely correlate with the effect on LOX. Recently, many synthetic chemists have focused their attention on the derivatives of NSAIDs with antioxidant activities. Zhang group[7] reported a series of conjugates of indomethacin with phenolic antioxidants which showed potent antioxidants *in vitro* enhanced antiinflammatory activity as well as reduced ulcerogenic potency.

Morpholine analogues have shown good anti-inflammatory and antioxidant activity. Kourounakis and co-workers[8] synthesized a variety of 2,6-di-*tert*-butylphenol, which are amides or amines of morpholine residues, some of which exhibited antiinflammatory, LOX inhibitory and antioxidant activities. Thus, we suppose that COX-2 inhibitors bearing morpholine derivatives could reduce the cardiovascular side effects after using COX-2 inhibitors for a long time.

Based on our research of NSAID naproxen derivatives[9] and phenethyl 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetate (**A**)[10], in this work, the morpholine moiety[11] was inserted into compound **A**, which has shown good antiinflammatory and antioxidant activity. That can be useful for the design of antiatherosclerotic agents (Scheme 1). We felt that the substituted 2-(2-arylmorpholino-4-yl)ethyl ester moiety might be of great value to explore deeply, as it is capable of inhibiting COX-2 selectively through forming additional hydrogen bonds by occupying the additional side pocket of COX-2 enzyme and reducing cardiovascular side effects by the antioxidant activity of morpholine moiety. These compounds were tested *in vitro* for the evaluation of their COX-2 selective inhibitory activities.

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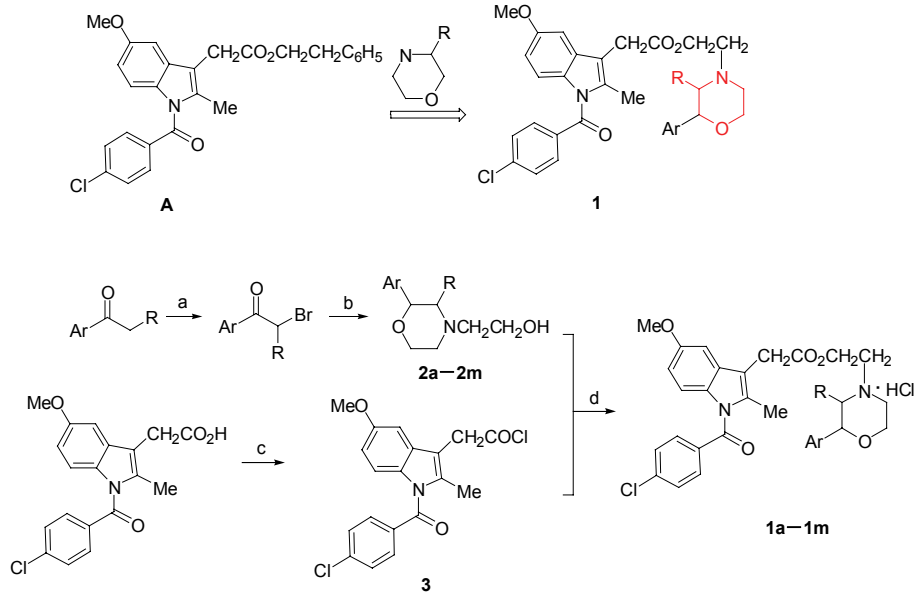
† Dedicated to Professor Jun Zhou on the occasion of his 80th birthday.

Scheme 1 Synthesis of 2-(2-arylmorpholino-4-yl)ethyl esters of indomethacin hydrochlorides



2a, **1a**: R=H, Ar=C_6H_5; **2b**, **1b**: R=H, Ar=4-$CH_3C_6H_4$; **2c**, **1c**: R=H, Ar=4-$C_2H_5C_6H_4$; **2d**, **1d**: R=H, Ar=4-$CH_3OC_6H_4$; **2e, 1e**: R=H, Ar=4-$(CH_3)_2CHOC_6H_4$; **2f**, **1f**: R=H, Ar=4-$CH_3(CH_2)_3OC_6H_4$; **2g**, **1g**: R=H, Ar=2,4-Cl_2-5-FC_6H_2; **2h**, **1h**: R=H, Ar=2-Cl-4-(4-ClC_6H_4O)C_6H_3; **2i**, **1i**: R=H, Ar=6-CH_3O-2-$C_{10}H_6$; **2j**, **1j**: R=H, Ar=6-CH_3O-5-Cl-2-$C_{10}H_5$; **2k**, **1k**: R=H, Ar=6-CH_3O-5-Br-2-$C_{10}H_5$; **2l, 1l**: R = CH_3, Ar=6-CH_3O-2-$C_{10}H_6$; **2m**, **1m**: R=CH_3, Ar=4-$C_6H_5CH_2OC_6H_4$

Reagents and conditions: (a) $CuBr_2$, EtOH, reflux, 6 h; (b) (i) $HN(CH_2CH_2OH)_2$, *N*-methylpyrrolidin-2-one (NMP), 60 °C, 2 h; (ii) HCOOH, 180 °C, 18 h; (c) $(COCl)_2$, CH_2Cl_2, 0 °C, 3 h; (d) (i) THF, Et_3N, r.t., 6 h; (ii) HCl(g), Et_2O then $(CH_3)_2CO$.

Experimental

All reagents and solvents were of commercial quality and used without further purification. Reactions were performed under the protection of nitrogen and were monitored by thin-layer chromatography (TLC) on silica gel plates, visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (200—400 mesh) using commercially available petroleum ether and ethyl acetate. Melting points (m.p.) were taken in open capillaries and uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker 400 MHz spectrometer. Chemical shifts (δ) are relative to tetramethylsilane multiplicities and given as s (singlet), d (doublet), t (triplet), and m (multiplet) as well as br (broad). Coupling constants (*J*) are given in Hz. Infrared spectra were recorded on an FT-IR spectrometer. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage ion-trap mass spectrometer. Microanalysis were performed using a C H N S/O analyzer. Elemental data are within ±0.2%. The following drugs and chemicals were purchased and used: sodium thioglycollate (DIFCO), calcium cation ionophore A23187, lipopolysaccharides (LPS), dimethyl sulfoxide (DMSO), Celecoxib (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).

Indomethacin acid chloride (3)

Indomethacin (3 mmol) and oxalyl chloride (1 mL) in dichloromethane (8 mL) were stirred at 0 ℃ for 3 h. The excess oxalyl chloride and dichloromethane were distilled off under reduced pressure, then the residue was washed with hexane (25 mL×3) and dried under vacuum to give **3** as pale yellow solid. Yield 92%, m.p. 126—128 ℃, which is in accordance with reference [12] values (Yield 95%, m.p. 125—127 ℃).

General procedure for the preparation of 2-(2-arylmorpholino-4-yl)ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochlorides 1a—1m

Indomethacin acid chloride prepared before was dissolved in 8 mL of tetrahydrofuran, then the solution of 2-(2-arylmorpholino-4-yl)ethanols **2a** — **2m** (4.5 mmol) as well as triethylamine (1 mL) was slowly added. The reaction mixture was stirred at room temperature for 6 h and filtered to remove triethylamine hydrochloride. The filtrate was distilled under reduced pressure to remove tretrahydrofuran, then the residue was dissolved with ethyl acetate and washed with aqueous sodium hydroxide (1.0 mol•L^{-1}), 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 hydro-

gen chloride, followed by filtration and residue was washed with acetone to give the hydrochlorides.

2-(2-Phenylmorpholino-4-yl)ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1a**): White solid, yield 72%; m.p. 184—186 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.35 (s, 3H, CH_3), 2.61 (br, 2H, C_4H_7NO-3,5-H_a), 3.21 (m, 2H, NCH_2), 3.35 (d, J=12 Hz, 1H, C_4H_7NO-5-H_e), 3.49 (d, J=11.2 Hz, 1H, C_4H_7NO-3-H_e), 3.80 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.93 (d, J=11.2 Hz, 1H, C_4H_7NO-6-H_a), 4.38 (t, J=12.8 Hz, 1H, C_4H_7NO-6-H_e), 4.68 (s, 2H, CH_2CO), 5.24 (d, J=10 Hz, 1H, C_4H_7NO-2-H), 6.68—7.68 (m, 12H, C_6H_5, C_6H_4, C_6H_3), 13.76 (s, 1H, NH); IR (KBr) ν: 3435, 3092, 1730, 1690, 1594, 1479, 1417, 1261, 1227, 1140 cm^{-1}; ESI-MS m/z: 546 (M^+—HCl). Anal. calcd for $C_{32}H_{34}Cl_2N_2O_5$: C 63.81, H 5.53, N 4.80; found C 63.79, H 5.52, N 4.75.

2-[2-(4-Methylphenylmorpholino-4-yl)ethyl [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1b**): White solid, yield 49%; m.p. 170—172 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.33 (s, 3H, CH_3), 2.35 (s, 3H, indole-2-CH_3), 2.59 (br, 2H, C_4H_7NO-3,5-H_a), 3.20 (m, 2H, NCH_2), 3.34 (d, J=12.4 Hz, 1H, C_4H_7NO-5-H_e), 3.46 (d, J = 12 Hz, 1H, C_4H_7NO-3-H_e), 3.80 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.91 (dd, J=3.2, 3.2 Hz, 1H, C_4H_7NO-6-H_a), 4.38 (t, J=12.0 Hz, 1H, C_4H_7NO-6-H_e), 4.68 (s, 2H, CH_2CO), 5.19 (d, J=9.2 Hz, 1H, C_4H_7NO-2-H), 6.68—7.68 (m, 11H, C_6H_5, C_6H_4, C_6H_3), 13.72 (s, 1H, NH); IR (KBr) ν: 3440, 2958, 1738, 1674, 1607, 1478, 1455, 1261, 1239, 1163 cm^{-1}; ESI-MS m/z: 560 (M^+—HCl). Anal. calcd for $C_{31}H_{32}Cl_2N_2O_5$: C 64.21, H 5.47, N 4.69; found C 64.33, H 5.44, N 4.68.

2-[2-(4-Ethylphenylmorpholino-4-yl)ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1c**): White solid, yield 22%; m.p. 186—189 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.21 (t, J=7.6 Hz, 3H, CH_3), 2.35 (s, 3H, indole-2-CH_3), 2.58—2.68 (m, 4H, $PhCH_2$, C_4H_7NO-3,5-H_a), 3.22 (m, 2H, NCH_2), 3.35 (d, J=10.8 Hz, 1H, C_4H_7NO-5-H_e), 3.48 (d, J=10.8 Hz, 1H, C_4H_7NO-3-H_e), 3.80 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.91 (d, J=12.4 Hz, 1H, C_4H_7NO-6-H_a), 4.38 (t, J=12.4 Hz, 1H, C_4H_7NO-6-H_e), 4.68 (s, 2H, CH_2CO), 5.20 (d, J = 10.8 Hz, 1H, C_4H_7NO-2-H), 6.68—7.68 (m, 11H, C_6H_5, C_6H_4, C_6H_3), 13.69 (s, 1H, NH); IR (KBr) ν: 3435, 2962, 1744, 1669, 1603, 1478, 1455, 1262, 1241, 1161 cm^{-1}; ESI-MS m/z: 574 (M^+—HCl). Anal. calcd for $C_{33}H_{36}Cl_2N_2O_5$: C 64.71, H 5.93, N 4.58; found C 64.78, H 5.99, N 4.56.

2-[2-(4-Methoxyphenylmorpholino-4-yl)ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1d**): White solid, yield 47%; m.p. 185—187 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 3H, CH_3), 2.59 (br, 2H, C_4H_7NO-3,5-H_a), 3.21 (br, 2H, NCH_2), 3.34 (d, J=11.2 Hz, 2H, C_4H_7NO-5-H_e), 3.44 (d, J=11.2 Hz, 2H, C_4H_7NO-3-H_e), 3.80 (s, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, indole-5-OCH_3), 3.91 (d, J=11.6 Hz, 1H, C_4H_7NO-6-H_a), 4.38 (t, J=8.8 Hz, 1H, C_4H_7NO-6-H_e), 4.68 (s, 2H, CH_2CO), 5.17 (d, J=10.4 Hz, 1H, C_4H_7NO-2-H), 6.86—7.68 (m, 11H, C_6H_4, C_6H_4, C_6H_3), 13.67 (s, 1H, NH); IR (KBr) ν: 3436, 2966, 2336, 1745, 1674, 1614, 1591, 1476, 1458, 1243, 1218, 1160, 1100 cm^{-1}; ESI-MS m/z: 576 (M^+—HCl). Anal. calcd for $C_{32}H_{34}Cl_2N_2O_6$: C 62.64, H 5.59, N 4.57; found C 62.59, H 5.65, N 4.59.

2-[2-(4-Isopropoxyphenylmorpholino-4-yl)ethyl [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1e**): White solid, yield 57%; m.p. 218—219 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.32 (d, J=6 Hz, 6H, $2CH_3$), 2.37 (s, 3H, CH_3), 2.61 (br, 2H, C_4H_7NO-3,5-H_a), 3.21 (br, 2H, NCH_2), 3.33 (d, J=11.2 Hz, 2H, C_4H_7NO-5-H_e), 3.44 (d, J = 11.6 Hz, 2H, C_4H_7NO-3-H_e), 3.81 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.90 (dd, J=2.8, 2.8 Hz, 1H, C_4H_7NO-6-H_a), 4.37 (t, J = 12.4 Hz, 1H, C_4H_7NO-6-H_e), 4.53 (heptet, 1H, $(CH_3)_2CHO$), 4.68 (s, 2H, CH_2CO), 5.15 (d, J=10.8 Hz, 1H, C_4H_7NO-2-H), 6.68—7.68 (m, 11H, C_6H_4, C_6H_4, C_6H_3), 13.69 (s, 1H, NH); IR (KBr) ν: 3081, 2987, 2289, 1742, 1671, 1634, 1599, 1480, 1449, 1322, 1251, 1147, 1085 cm^{-1}; ESI-MS m/z: 604 (M^+—HCl). Anal. calcd for $C_{34}H_{38}Cl_2N_2O_6$: C 63.81, H 5.53, N 4.80; found C 63.88, H 5.40, N 4.79.

2-[2-(4-Butoxyphenylmorpholino-4-yl)ethyl [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1f**): White solid, yield 46%; m.p. 179—180 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.97 [t, J=6.8 Hz, 3H, $CH_3(CH_2)_3$], 1.48 (m, 2H, CH_3CH_2), 1.75 (m, 2H, $CH_3CH_2CH_2$), 2.36 (s, 3H, CH_3), 2.60 (br, 2H, C_4H_7NO-3,5-H_a), 3.21 (br, 2H, NCH_2), 3.33 (d, J=12.0 Hz, 1H, C_4H_7NO-5-H_e), 3.44 (d, J = 11.6 Hz, 1H, C_4H_7NO-3-H_e), 3.80 (s, 2H, $C_3H_7CH_2O$), 3.85 (s, 3H, indole-5-OCH_3), 3.92 (s, 2H, OCH_2), 3.94 (d, J=10.8 Hz, 1H, C_4H_7NO-6-H_a), 4.37 (t, J = 12.4 Hz, 1H, C_4H_7NO-6-H_e), 4.68 (s, 2H, CH_2CO), 5.15 (d, J=10.4 Hz, 1H, C_4H_7NO-2-H), 6.68—7.22 (m, 11H, C_6H_4, C_6H_4, C_6H_3), 13.65 (s, 1H, NH); IR (KBr) ν: 3098, 2948, 2234, 1752, 1672, 1593, 1525, 1474, 1411, 1332, 1252, 1149, 1102 cm^{-1}; ESI-MS m/z: 618 (M^+—HCl). Anal. calcd for $C_{35}H_{40}Cl_2N_2O_6$: C 64.12, H 6.15, N 4.27; found C 64.15, H 6.12, N 4.20;

2-[2-(2,4-Dichloro-5-fluorophenyl)morpholino-4-yl]-ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1g**): White solid, yield 43%; m.p. 171—174 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 3H, CH_3), 2.59 (br, 2H, C_4H_7NO-3,5-H_a), 3.21—3.37 (m, 4H, C_4H_7NO-3,5-H_e, NCH_2), 3.81 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.95 (d, J=12 Hz, 1H, C_4H_7NO-6-H_a), 4.45 (s, 1H, C_4H_7NO-6-H_e), 4.69 (s, 2H, CH_2CO), 5.52 (d, J=8.0 Hz, 1H, C_4H_7NO-2-H), 6.68—7.68 (m, 9H, C_6H_4, C_6H_3, C_6H_2), 14.06 (s, 1H, NH); IR (KBr) ν: 3436, 2928, 2336, 1736, 1679, 1612, 1455, 1389, 1257, 1237, 1167, 1144 cm^{-1}; ESI-MS m/z: 632 (M^+—HCl—1). Anal. calcd for $C_{31}H_{29}Cl_4N_2O_5$: C 55.44, H 4.36, N 4.18; found C 55.58, H 4.31, N 4.19.

2-[(2-Chloro-4-(4-chlorophenoxy)phenyl)-morpholino-4-yl]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-

methyl-1*H*-indol-3-acetate hydrochloride (**1h**): White solid, yield 33%; m.p. 166—168 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 3H, CH_3), 2.51 (br, 2H, C_4H_7NO-3,5-H_a), 3.21 (br, 2H, NCH_2), 3.35 (d, J=10.4 Hz, 1H, C_4H_7NO-5-H_e), 3.65 (d, J=12.8 Hz, 1H, C_4H_7NO-3-H_e), 3.81 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.94 (d, J=12.4 Hz, 1H, C_4H_7NO-6-H_a), 4.45 (t, J=11.6 Hz, 1H, C_4H_7NO-6-H_e), 4.69 (s, 2H, CH_2CO), 5.53 (d, J=10.4 Hz, 1H, C_4H_7NO-2-H), 6.68—7.68 (m, 14H, C_6H_4, C_6H_4, C_6H_3, C_6H_3), 14.03 (s, 1H, NH); IR (KBr) ν: 3435, 2952, 2435, 1721, 1685, 1608, 1591, 1480, 1457, 1400, 1272, 1247, 1122, 1092 cm^{-1}; ESI-MS *m*/*z*: 708 (M^+−HCl+1). Anal. calcd for $C_{37}H_{34}Cl_4N_2O_6$: C 59.69, H 4.60, N 3.76; found C 59.78, H 4.56, N 3.77.

2-[2-(6-Methoxynaphthalen-2-yl)morpholino-4-yl]-ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1i**): White solid, yield 30%; m.p. 101—102 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 3H, CH_3), 2.47 (br, 2H, C_4H_7NO-3,5-H_a), 2.92—3.28 (m, 4H, NCH_2, C_4H_7NO-3,5-H_e), 3.73 (s, 2H, OCH_2), 3.83 (s, 3H, indole-5-CH_3O), 3.91 (s, 3H, CH_3O), 3.97 (d, J=8 Hz, 1H, C_4H_7NO-6-H_a), 4.01 (br, 1H, C_4H_7NO-6-H_e), 4.48 (s, 2H, CH_2CO), 4.91 (m, 3H, OCH_2, C_4H_7NO 2-H), 6.64—7.72 (m, 13H, $C_{10}H_6$, C_6H_4, C_6H_3), 13.49 (s, 1H, NH); IR (KBr) ν: 3439, 2992, 2929, 1744, 1676, 1603, 1477, 1467, 1360, 1324, 1291, 1236, 1215, 1184, 1089, 1072, 1036 cm^{-1}; ESI-MS *m*/*z*: 626 (M^+−HCl). Anal. calcd for $C_{36}H_{36}Cl_2N_2O_6$: C 65.16, H 5.47, N 4.22; found C 65.13, H 5.42, N 4.28.

2-[2-(6-Methoxy-5-chloronaphthalen-2-yl) morpholino-4-yl]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1j**): White solid, yield 33%; m.p. 225—227 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.37 (s, 3H, CH_3), 2.66 (br, 2H, C_4H_7NO-3,5-H_a), 3.31—3.55 (m, 4H, NCH_2, C_4H_7NO-3,5-H_e), 3.81 (s, 2H, OCH_2), 3.85 (s, 3H, indole-5-CH_3O), 3.99 (br, 1H, C_4H_7NO-6-H_a), 4.05 (s, 3H, CH_3O), 4.46 (br, 1H, C_4H_7NO-6-H_e), 4.71 (br, 2H, CH_2CO), 5.44 (br, 1H, C_4H_7NO-2-H), 6.68—8.22 (m, 12H, $C_{10}H_5$, C_6H_4, C_6H_3), 13.78 (s, 1H, NH); IR (KBr) ν: 3436, 2958, 2326, 1746, 1669, 1604, 1479, 1455, 1356, 1340, 1279, 1243, 1228, 1162, 1140, 1093, 1072, 1036 cm^{-1}; ESI-MS *m*/*z*: 667 (M^+−HCl). Anal. calcd for $C_{36}H_{35}Cl_3N_2O_6$: C 61.94, H 5.05, N 4.01; found C 61.98, H 5.11, N 3.94.

2-[2-(6-Methoxy-5-bromonaphthalen-2-yl)-morpholino-4-yl]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1k**): White solid, yield 45%; m.p. 229—230 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 3H, CH_3), 2.66 (br, 2H, C_4H_7NO-3,5-H_a), 3.28—3.56 (m, 4H, NCH_2, C_4H_7NO-3,5-H_e), 3.81 (s, 2H, OCH_2), 3.85 (s, 3H, indole-5-CH_3O), 3.98 (br, 1H, C_4H_7NO-6-H_a), 4.04 (s, 3H, CH_3O), 4.46 (br, 1H, C_4H_7NO-6-H_e), 4.71 (br, 2H, CH_2CO), 5.43 (br, 1H, C_4H_7NO-2-H), 6.68—8.22 (m, 12H, $C_{10}H_5$, C_6H_4, C_6H_3), 13.80 (s, 1H, NH); IR (KBr) ν: 3460, 2957, 2326, 1742, 1668, 1606, 1479, 1456, 1357, 1327, 1274, 1163, 1142, 1095, 1068, 1037 cm^{-1}; ESI-MS *m*/*z*: 706 (M^+−HCl). Anal. calcd for $C_{36}H_{35}BrCl_2N_2O_5$: C 58.24, H 4.75, N 4.75; found C 58.21, H 4.83, N 4.79.

2-[3-Methyl-2-(6-methoxynaphthalen-2-yl)-morpholino-4-yl]ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1l**): White solid, yield 22%; m.p. 145—147 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.27 (d, J=6.4 Hz, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.72 (br, 1H, C_4H_7NO-3-H), 3.08 (br, 2H, NCH_2), 3.31 (d, J=11.2 Hz, 1H, C_4H_7NO-5-H_a), 3.67 (br, 1H, C_4H_7NO-5-H_e), 3.77 (dd, J = 10.4, 2.0 Hz, 1H, C_4H_7NO-6-H_a), 3.81 (s, 2H, OCH_2), 3.86 (s, 3H, indole-5-CH_3O), 3.93 (s, 3H, CH_3O), 4.46 (br, 1H, C_4H_7NO-6-H_e), 4.66 (s, 2H, CH_2CO), 5.01 (br, 2H, C_4H_7NO-2-H), 6.69—7.75 (m, 13H, $C_{10}H_6$, C_6H_4, C_6H_3), 13.47 (s, 1H, NH); IR (KBr) ν: 3309, 2953, 2630, 1686, 1635, 1609, 1486, 1452, 1373, 1267, 1223, 1174, 1126, 1080, 1026, 979 cm^{-1}; ESI-MS *m*/*z*: 660 (M^+−HCl). Anal. calcd for $C_{31}H_{32}Cl_2N_2O_5$: C 65.58, H 5.65, N 4.13; found C 65.54, H 5.69, N 4.10.

2-[3-Methyl-2-(4-benzyloxyphenyl)morpholino-4-yl]-ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochloride (**1m**): White solid, yield 17%; m.p. 122—125 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.21 (br, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.68 (br, 1H, C_4H_7NO-3-H), 2.95 (br, 2H, NCH_2), 3.15 (br, 1H, C_4H_7NO-5-H_a), 3.70 (br, 1H, C_4H_7NO-5-H_e), 3.83—3.86 (br, 5H, OCH_2, CH_3O), 3.93 (s, 1H, C_4H_7NO-6-H_a), 4.41 (br, 1H, C_4H_7NO-6-H_e), 4.65 (s, 2H, CH_2CO), 4.82 (br, 1H, C_4H_7NO-2-H), 5.06 (s, 2H, $PhCH_2O$), 6.68—7.68 (m, 16H, C_6H_5, C_6H_4, C_6H_4, C_6H_3), 13.37 (s, 1H, NH); IR (KBr) ν: 3339, 2932, 2609, 1737, 1681, 1611, 1514, 1477, 1458, 1239, 1175, 1146, 1089 cm^{-1}; ESI-MS *m*/*z*: 640 (M^+−HCl). Anal. calcd for $C_{31}H_{32}Cl_2N_2O_5$: C 66.57, H 5.73, N 3.98; found C 66.46, H 5.76, N 3.95

COX-1/2 inhibitory assay

Peritoneal macrophages were obtained from mice that had been euthanized by cervical dislocation. The peritonea of the animals were surgically exposed using a midline incision. An experimental group of mice had been administered an intraperitoneal injection of 2 mL of 30 mg/mL sodium thioglycollate 4 d prior to the sacrifice. Following 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 for twice.

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

The mouse peritoneal macrophages were seeded in 48-well plates (1×10^9 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: (1) negative control group of DMSO (control); (2) A23187 (final concentration 1

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μmol•L^{-1}); (3) A23187＋**10a**—**10g** (5 μmol•L^{-1}L); (4) A23187＋celecoxib (1 μmol•L^{-1}). Experiments were performed at least three times in triplicate. The peritoneal macrophages, drugs or solvents were incubated for 1 h at 37 ℃ with 5% CO_2, then A23187 was added (final concentration 1 μmol•L^{-1}) 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 processed according to the following four groups: (1) Negative control group of DMSO (control); (2) LPS (final concentration 1 mg/L); (3) LPS＋**10a**—**10g** (5 μmol•L^{-1}); (4) LPS＋celecoxib (1 μmol•L^{-1}). The peritoneal macrophages, drugs or solvents were also incubated for 1 h at 37 ℃ 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 ^3H.

Results and Discussion

Preparation of the intermediate 2-(2-arylmorpholino-4-yl)ethanols **2a**—**2m** followed the method reported before by us,[9,13] arylalkylones were brominated via copper(II) bromide in the presence of ethanol as solvent affording *α*-phenacylbromides. Reaction of phenacylbromides with 2,2'-azanediyl-diethanol gave hemiketals, formic acid (88%) was added, then intermediates **2a**—**2m** formed via a Leuckart-Wallach reaction.[14]

Then indomethacin acid chloride (**3**) was reacted with 2-(2-phenylmorpholino-4-yl)ethanol (**2a**) without purification in the presence of tetrahydrofuran (THF) and triethylamine to obtain the ester as an oily product, which was converted into its hydrochloride derivative **1a** as white solid. The other products **1b**—**1m** were obtained in a similar way by reacting their respective esters with hydrochloride. All the compounds were characterized by their spectra data.

All of the new compounds synthesized were tested *in vitro* initially at 10.0 μmol•L^{-1} for selectivity and potency against human COX-1 and COX-2 enzymes. On the basis of their *in vitro* efficacy, selected compounds **1a**—**1g** were tested against COX-1 at 0.1, 1.0, 10.0 and 100 μmol•L^{-1}, respectively, while against COX-2 at 0.01, 0.1, 1.0 and 10.0 μmol•L^{-1}, respectively. Celecoxib was used as reference compound for the *in vitro* assay. The concentration of the selected/promising compounds for 50% inhibition of COX-1 and COX-2 (IC_{50}) was calculated using the software (SPSS). The results are shown in Table 1.

Indomethacin is a non-selective inhibitor of COX isozymes [COX-2 SI＜1, the ratio of IC_{50}(COX-1) to IC_{50}(COX-2)].[15-17] Biological evaluation of the title compounds showed that the conversion of the free acid group of non-selective inhibitor indomethacin to the 2-(2-arylmorpholino-4-yl)ethyl ester gives rise to compounds possessing various degrees of COX-2 selectivity. Nonsubstituted phenyl derivative **1a** which showed weak COX-1 and COX-2 inhibitory activity differs from other compounds with substituents on the phenyl ring. The results are similar with the reported unsubstituted benzyl esters of 4- or 6-chloroindomethacin which also showed weak inhibitory activity.[18]

Table 1 Selected examples of COX inhibitory activity and selectivity

Compd.	Selectivity index (COX-1/COX-2)	IC_{50}/(μmol•L^{-1})	
		COX-2	COX-1
1a	＞1.1	89.4	＞100
1b	49	0.076	3.72
1c	50	0.031	1.56
1d	30	0.071	2.13
1e	58	0.13	7.52
1f	182	0.084	15.3
1g	358	0.038	13.6
Celecoxib	214	0.05	10.7

Replacement of phenyl by 4-methylphenyl group (**1b**, IC_{50}＝0.076 μmol•L^{-1}) increased the selectivity and potency further against COX-2. 4-Ethylphenyl derivative (**1c**, IC_{50}＝0.031 μmol•L^{-1}) showed greater COX-2 inhibitory activity than 4-methylphenyl derivative (**1b**) probably because the larger group ethylphenyl in size possessed the side pocket of COX-2 isoenzyme more easily. A variety of alkoxylphenyl moieties (**1d**—**1f**) were examined and all induced moderate to good COX-2 selectivity. Among the alkoxyl derivatives, 4-(*n*-BuO)phenyl derivative (**1f**, IC_{50}＝15.3 μmol•L^{-1}) showed least COX-1 inhibitory activity and greatest COX-2 selective inhibitory activity (SI 182), which is comparative with celecoxib (SI 214). Compared with the alkyl replacement derivatives, the alkoxyl derivatives showed higher selectivity with little variation of COX-2 inhibitory. The reason may be that: (1) the alkoxyl replacement with bigger size than alkyl group leading to greater steric hindrance hindered the binding with the side pocket of COX-2; (2) the hydrogen bonds between alkoxyl and amino acid residues strengthened the binding, the proportion of the two sides led to the different performances of **1d**—**1f**. The least COX-2 inhibitory activity of **1e** (IC_{50}＝0.13 μmol•L^{-1}) may be because that 4-isopropoxyphenyl replacement caused a too big steric hindrance which hindered the binding with the side pocket. It means that only the replacement with proper size could enhance the binding.

Remarkably, selectivity was observed for strong electron-withdrawing group such as fluoro and chloro atoms, compound **1g** possessing 2,4-dichloro-5-fluorophenyl group showed better COX-2 inhibitory activity and selectivity over celecoxib. We suppose that strong

hydrogen bonds were formed between fluoro, chloro atoms and amino acid residues in the side pocket of COX-2 enzyme.

This study revealed that the replacement of the phenyl could lead to increased selectivity and potency further against COX-2. The steric hindrance produced by phenyl replacement hindered the binding with the COX-1 and promoted the binding with COX-2, however, too big replacement (4-isopropoxyphenyl) hindered the binding with the side pocket too. The fluoro, chloro atoms in the replacement could strongly strengthen the selectivity and potency further against COX-2. Totally, the selectivity and potency against COX-2 were determined by the steric hindrance caused by the replacement, and the hydrogen bonds formed between the replacement and the amino acids residues as well.

Conclusions

With the aim of developing potent but moderately selective COX-2 inhibitors and increasing antioxidant activity, we have shown that in addition to the earlier report of converting NSAID indomethacin into simple esters, it can also be transformed into COX-2 inhibitors via converting the acid moiety to 2-(2-arylmorpholino-4-yl)ethyl esters of indomethacin which were synthesized to determine their ability to inhibit the COX-1 and COX-2 isozymes. *In vitro* enzyme inhibition studies indicated that some of the compounds synthesized showed moderate to good selective COX-2 inhibitory activity (SI 30—358), which can reduce GI side effects generated by NSAID indomethacin. Meanwhile, the morpholine moiety contained in the side chain of the esters endows these compounds with antioxidant activity, which could limit the cardiovascular side effects caused for its selective COX-2 inhibitory in minimum extent. The cardiovascular side effects caused for its selective COX-2 inhibitory activity could also be limited in minimum extent. Therefore, the novel 2-(2-arylmorpholino-4-yl) ethyl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate hydrochlorides developed by us seem to have advantages over other COX-2 inhibitors. Compounds **1f** and **1g** were identified as promising candidates in this class and their antioxidant activities were under studying.

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