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RESEARCH ARTICLE

Synthesis and *in-silico* studies of some diaryltriazole derivatives as potential cyclooxygenase inhibitors

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Abstract The synthesis of several 4-phenyl-5-pyridin-4-yl-2,3-dihydro-3H-1,2,4-triazole-3-thiones possessing N-2 Mannich bases or S-alkyl substituents, is reported. Several of them exhibited a low nanomolar COX enzyme inhibition activity. Most of the compounds showed inhibition of edema was similar to that evoked by celocoxib in animal model. Molecular docking studies of the compounds into the binding sites of COX-1 and COX-2 allowed us to shed light on the binding mode of these novel COX inhibitors.

Keywords Triazole · Cyclooxygenase · Antiinflammatory · Surflex-Dock

Introduction

Through the path of prostaglandin–thromboxane synthesis, the transformation of arachidonic acid to prostaglandin H2 (PGH2) is catalyzed by cytosolic prostaglandin G/H synthase, more commonly known as cyclooxygenase (COX). Prostaglandin H2 is an unstable intermediate and is further converted to one of many prostanoids, such as prostacyclin (PGI2) and thromboxane A2 (TXA2), by tissue-specific isomerases. The biosynthesis of these prostaglandins is practically in all tissues of the human body, eliciting a variety of pharmacological effects, some of them beneficial, as support of renal and platelet functions, gastrointestinal protection, and other nonbeneficial as pain, fever and other symptoms associated with the inflammatory response. Since 1987, it has been established that COX enzyme exists in two isoforms (Tanaka et al. 1987), and are encoded by separate genes on different chromosomes. Cyclooxygenase inhibition formed the basis for the success of non-steroidal anti-inflammatory drugs (NSAIDs) in treating a variety of pain syndromes. The drawback, however, was that every year, 2–4 % of patients taking NSAIDs suffered from symptomatic gastrointestinal ulcers and their complications (Silverstein et al. 2000). As the scientific research behind the COX enzyme progressed, it became apparent that COX-2 inhibition mediated the anti-inflammatory effects of NSAIDs, whereas COX-1 inhibition was responsible for the adverse effects on the gastrointestinal tract. It therefore became reasonable to assume that inhibiting COX-2 selectively would result in the same anti-inflammatory benefits that non-selective NSAIDs provided but with fewer gastrointestinal side effects. This enforced the interest of pharmaceutical industries in manufacturing new analgesic and anti-inflammatory medications known as selective COX-2 inhibitors or coxibs (Penning et al. 1997; Riendeau et al. 2001; Ranatunge et al. 2004). A selective COX-2 inhibitor allows the desired synthesis of cytoprotective prostaglandins, in conjunction with a simultaneous inhibition of proinflammatory prostaglandin synthesis, thereby reducing dyspepsia and ulceration (Meade et al. 1993). However, emerging evidence suggests that adverse reactions such as gastrointestinal irritations or ulceration and renal liabilities are associated with prolonged use of COX-2 selective inhibitors. The adverse reactions have been attributed, at least in part, to COX-1 inhibition

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occurring with long-term exposure or at higher doses (Wolfe et al. 1999). COX-2 selective inhibitors are also known to suppress synthesis of prostacyclin, a potent vasodilator, gastroprotectant, and platelet inhibitor, via inhibition of endothelial COX-2. COX-2 selective inhibitors do not inhibit production of thromboxane, a vasoconstrictor, and promoter of platelet aggregation, which is synthesized in platelets by COX-1 (Catella-Lawson and Crofford 2001; Mukherjee et al. 2001). Therefore, COX-2 inhibitors intrinsically lack anti-thrombotic activity, and some cardiovascular liabilities have been associated preclinically with them (deGaetano et al. 2003). Thus, there is still a need for novel, selective, and potent COX-2 inhibitors with an improved profile compared to current COX-2 inhibitors (Ranatunge et al. 2004). Diaryl heterocycles, and other central ring pharmacophore templates, have been extensively studied as cyclooxygenase inhibitors. All these tricyclic molecules possess 1,2-diaryl substitution on a central four-, five-, or six-membered ring system such as cyclobutenone, pyrazole, 2-(5H)-furanone, isoxazole, pyridine, or thiazolidinedione respectively (Talley et al. 2000; Penning et al. 1997; Riendeau et al. 2001; AbdelMoallem et al. 2011; Zarghi et al. 2011; Ali et al. 2007). Recently, a novel class of 6-alkylthio-substituted six-membered lactone (pyrane-2-one) rings has been designed and exhibited very good in vitro COX-2 inhibitory potency and selectivity (Praveen et al. 2003). Furthermore, differently substituted 1,2,4-triazole-3-thione derivatives exhibited anti-inflammatory activity (Labanauskas et al. 2001; Tozkoparan et al. 2005). Structure based studies have been performed to identify binding modes and important interactions of triaryl rings at the COX-2 active site (Plount-Price and Jorgensen 2000). The triaryl ring moiety either containing a para-sulfonyl group (Talley et al. 2000; Penning et al. 1997; Riendeau et al. 2001; AbdelMoallem et al. 2011; Zarghi et al. 2011; Ali et al. 2007) or not containing a para-sulfonyl group (Sui et al. 2000; Dannhardt and Laufer 2000; Moreau et al. 2006) has been recognized as a pharmacophore for selective COX-2 inhibition.

Considering these results and as part of our ongoing program to design novel selective COX-2 inhibitors, we describe herein the design, synthesis, and biological evaluation of a novel diverse group of 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole derivatives incorporating 2-alkyl(aryl)aminomethyl or 3-alkkylthio moiety at the central triazole ring.

Materials and methods

Chemistry

Melting points were determined on Barnstead 9001 Electrothermal melting point apparatus using open capillary tubes and are uncorrected. IR spectra were obtained, as KBr discs, on a Perkin-Elmer FT-IR spectrophotometer at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. The data are given in ν_{max} (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded in either DMSO-d_6 or $CDCl_3$ on a Bruker NMR spectrophotometer operating at 500 MHz for ^1H and 125.76 MHz for ^{13}C; the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) used as internal standard. Mass spectra were taken on a Varian320-MS spectrometer at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. Mass spectral data were given as *m/z* (intensity%). Elemental analysis was performed on a Perkin-Elmer CHNSO analyzer, model no. 2400. Monitoring of reactions and checking of purity of the final products were carried out by thin layer chromatography (TLC) using silica gel precoated aluminum sheets (60 F254, Merck) and visualization with ultraviolet light (UV) at 365 and 254 nm. Isonicotinic acid hydrazide (**1**) and phenylisothiocyanate (**2**) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). 2-Isonicotinoyl-*N*-phenylhydrazinecarbothioamide (**3**) and 4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**4**) were synthesized according reported procedures (Bayrak et al. 2009). Molecular modeling was carried out using Surflex-Dock module within Sybyl 8.1.1 package (2006) (Tripos Inc., St. Louis, USA). The program operated under "Linux_OS2x" operating system installed on HPxw8400 workstation at laboratory of drug design, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan.

Ethyl[4-phenyl-5-pyridin-4-yl-3-thioxo-2,4-dihydro-3H-1,2,4-triazol-2-yl]carboxylate ***5***

A mixture of compound **4** (2.54 g, 10 mmol), 0.9 g anhydrous sodium carbonate and the ethyl chloroformate (10 mmol) in DMF (5 mL) was refluxed for 7 h. The reaction mixture was cooled then poured into ice-cold water. The solid formed was filtered off and recrystallized from acetic acid to give compounds **5**. Yield 2.7 g, 82 %, m.p. 231–232 °C; IR (KBr) 3057, 1770, 1654, 1636, 1298, 828, 741, 697 cm^{-1}; ^1H NMR (DMSO-d_6, δ ppm): 1.6 (3H, t, CH_3), 4.5 (2H, q, CH_2–O), 7.0–7.1 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.1–7.25 (2H, m, C_3H and C_5H of phenyl), 7.4 (2H, d, C_3H and C_5H of pyridine), 8.5 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.65; H, 4.53; N, 17.08; S, 9.61.

2-Hydroxymethyl-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione ***6***

A mixture of **4** (2.54 g, 10 mmol) and formalin (2 mL, 25 mmol) in water (10 mL) was heated under reflux for 5 min, (Wujec et al. 2003; Wujec and Paneth 2008) cooled

to room temperature and filtered. The solid was washed with cold water and dried to produce **6**. The crude product was recrystallized from ethanol. Yield 2.2 g, 78 %, m.p. 277–278 °C; IR (KBr) 3450, 3116, 2851, 1654, 1270, 830, 741, 695 cm^{-1}; ^1H NMR (DMSO-d_6, δ ppm): 3.3–3.8 (1H, broad s, OH), 5.7 (2H, s, NCH_2O), 7.05–7.25 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.25–7.4 (2H, m, C_3H and C_5H of phenyl), 7.5 (2H, d, C_3H and C_5H of pyridine), 8.5 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{14}H_{12}N_4OS$: C, 59.14; H, 4.25; N, 19.70; S, 11.28. Found: C, 58.92; H, 4.05; N, 19.40; S, 11.35.

2-Substituted-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole-3-thiones ***7–12***

To a solution of compound **4** (2.54 g, 10 mmol) in DMF (10 mL), formaldehyde (37 %, 1.55 mL) and an appropriate amine (10 mmol) were added and the mixture was stirred at room temperature for 3 h. Then, excess amount of pure water was added to this solution and the mixture was kept overnight in cold. The resulting solid separated was collected by filtration, washed with water, recrystallized from ethanol to yield the title compounds.

2-Dimethylaminomethyl-4-phenyl-5-pyridin-4-yl-2, 4-dihydro-3H-1,2,4-triazole-3-thione ***7***

Yield 2.52 g, 78 %, m.p. 173–174 °C; IR (KBr) 3065, 2973, 1654, 1279, 832, 752, 697 cm^{-1}; ^1H NMR, ($CDCl_3$, δ ppm): 2.7 (6H, s, $(CH_3)_2N$), 5.3 (2H, s, NCH_2N), 7.2–7.35 (3H, m, C_2H and C_6H of phenyl), 7.35–7.55 (3H, m, C_3H, C_4H and C_5H of phenyl), 7.6 (2H, d, C_3H and C_5H of pyridine), 8.6 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{16}H_{17}N_5S$: C, 61.71; H, 5.50; N, 22.49; S, 10.30. Found: C, 61.53; H, 5.74; N, 22.21; S, 10.62.

2-Diethylaminomethyl-4-phenyl-5-pyridin-4-yl-2, 4-dihydro-3H-1,2,4-triazole-3-thione ***8***

Yield 2.79 g, 83 %, m.p. 216–217 °C; IR (KBr) 3076, 2965, 1654, 1279, 835, 748, 695 cm^{-1}; ^1H NMR (DMSO-d_6, δ ppm): 1.7 (6H, t, $2CH_3$), 2.6 (4H, q, $2CH_2N$), 5.7 (2H, s, NCH_2N), 7.0–7.2 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.2–7.4 (2H, m, C_3H and C_5H of phenyl), 7.6 (2H, d, C_3H and C_5H of pyridine), 8.6 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{18}H_{21}N_5S$: C, 63.69; H, 6.24; N, 20.63; S, 9.45. Found: C, 63.85; H, 6.33; N, 20.42; S, 9.11.

4-Phenyl-5-pyridin-4-yl-2-pyrrolidinylmethyl-2, 4-dihydro-3H-1,2,4-triazole-3-thione ***9***

Yield 2.96 g, 90 %, m.p. 139 °C; IR (KBr) 3116, 2836, 1654, 1636, 1270, 832, 742, 695 cm^{-1}; ^1H NMR, (DMSO-d_6, δ ppm): 1.8 (4H, t, CH_2CH_2), 3.0 (4H, t, CH_2N CH_2), 5.7 (2H, s, NCH_2N), 7.0–7.2 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.2–7.4 (2H, m, C_3H and C_5H of phenyl), 7.6 (2H, d, C_3H and C_5H of pyridine), 8.6 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{18}H_{19}N_5S$: C, 64.07; H, 5.68; N, 20.75; S, 9.50. Found: C, 64.21; H, 5.82; N, 20.58; S, 9.34.

N-Methyl-N-[(4-phenyl-5-pyridin-4-yl-3-thioxo-2, 4-dihydro-3H-1,2,4-triazol-2-y)methyl]-2-aminoethanol ***10***

Yield 2.52 g, 73 %, m.p. 173–174 °C; IR (KBr) 3385, 3104, 2850, 1654, 1270, 868, 774, 694 cm^{-1}; ^1H NMR, ($CDCl_3$, δ ppm): 1.8 (1H, broad s, OH), 2.8 (3H, s, NCH_3), 3.0 (2H, t, NCH_2), 4.0 (2H, t, CH_2O), 5.8 (2H, s, NCH_2N), 7.20–7.25 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.27–7.38 (2H, m, C_3H and C_5H of phenyl), 7.6 (2H, d, C_3H and C_5H of pyridine), 8.6 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{17}H_{19}N_5OS$: C, 59.80; H, 5.61; N, 20.51; S, 9.39. Found: C, 60.01; H, 5.50; N, 20.32; S, 9.25.

2-[(4-Bromophenylamino)methyl]-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione ***11***

Yield 3.19 g, 75 %, m.p. 208–209 °C; IR (KBr) 3032, 2944, 1654, 1636, 1276, 833, 807, 740 cm^{-1}; ^1H NMR, ($CDCl_3$, δ ppm): 5.5 (1H, broad s, NH), 5.7 (2H, s, NCH_2N), 6.9–7.0 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.15–7.3 (2H, m, C_3H and C_5H of phenyl), 7.3 (2H, d, C_2H and C_6H of *p*-bromophenyl), 7.4 (2H, d, C_3H and C_5H of *p*-bromophenyl), 7.6 (2H, d, C_3H and C_5H of pyridine), 8.6 (2H, d, C_2H and C_6H of pyridine). ^{13}C NMR, ($CDCl_3$, δ ppm): 65.44 (NCH_2N), 111.52 (C), 115.94 (2CH), 121.69 (2CH), 128.06 (CH), 130.12 (2CH), 130.46 (2CH), 132.19 (2CH), 132.86 (C), 134.10 (C), 143.73 (C), 146.86 (2CH), 150.10 (triazole C-3), 169.51 (triazole C-5). Anal. calcd. for $C_{20}H_{16}BrN_5S$: C, 54.80; H, 3.68; N, 15.98; S, 7.32. Found: C, 54.69; H, 3.51; N, 15.83; S, 7.12.

2-[(4-Chlorobenzylamino)methyl]-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole-3-thione ***12***

Yield 2.89 g, 72 %, m.p. 222–223 °C; IR (KBr) 3052, 2928, 1654, 1636, 1274, 828, 798, 697 cm^{-1}; ^1H NMR, ($CDCl_3$, δ ppm): 1.9 (1H, broad s, NH), 4.5 (2H, s, benzylic CH_2), 5.8 (2H, s, NCH_2N), 7.12–7.17 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.21–7.26 (2H, m, C_3H and C_5H of phenyl), 7.29 (2H, d, C_2H and C_6H of *p*-chlorophenyl), 7.38 (2H, d, C_3H and C_5H of *p*-chlorophenyl), 7.55 (2H, d, C_3H and C_5H of pyridine), 8.59 (2H, d, C_2H and C_6H of pyridine). ^{13}C NMR, ($CDCl_3$, δ ppm): 55.23 (benzylic CH_2N), 68.11 (NCH_2N), 121.56 (2CH), 128.06 (CH), 128.20 (2CH), 129.63 (2CH), 130.05 (2CH), 130.34 (2CH),

132.77 (C), 132.86 (C), 134.38 (C), 136.86 (C), 146.99 (2CH), 150.41 (triazole C-3), 170.09 (triazole C-5). Anal. calcd. for $C_{21}H_{18}ClN_5S$: C, 61.83; H, 4.45; N, 17.17; S, 7.86. Found: C, 61.60; H, 4.57; N, 17.01; S, 8.03.

*3-Substituted-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazoles 13-**16***

To a solution of compound **4** (2.54 g, 10 mmol) in absolute ethanol, 1 equiv. of sodium was added and the mixture was stirred at room temperature for 30 min. Then, the appropriate alkyl halide (20 mmol) was added and refluxed for 4 h. After evaporating the solvent under reduced pressure a solid appeared. The solid was recrystallized from ethanol/water (1:1) to obtain target compound.

*3-Methylthio-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole **13***

Yield 1.52 g, 61 %, m.p. 145–146 °C (reported 168–170 °C) (Bayrak et al. 2009); IR (KBr) 3035, 2957, 1654, 1637, 1265, 836, 775, 693 cm^{-1}; ^1H NMR, (DMSO-d_6, δ ppm): 2.75 (1H, s, SCH_3), 7.27–7.29 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.32–7.34 (2H, m, C_3H and C_5H of phenyl), 7.58 (2H, d, C_3H and C_5H of pyridine), 8.58 (2H, d, C_2H and C_6H of pyridine). ^{13}C NMR, (DMSO-d_6, δ ppm): 14.59 (CH_3–S), 121.50 (2CH), 127.11 (CH), 130.38 (2CH), 130.55 (2CH), 133.68 (1CH), 134.32 (1CH), 149.96 (triazole C-3), 152.51 (2CH), 155.47 (triazole C-5). Anal. calcd. for $C_{14}H_{12}N_4S$: C, 62.66; H, 4.51; N, 20.88; S, 11.95. Found: C, 62.51; H, 4.64; N, 20.72; S, 12.10.

*4-Phenyl-3-(prop-2-ynylthio)-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole **14***

Yield 2.26 g, 79 %, m.p. 151–152 °C; IR (KBr) 3321, 2974, 1654, 1637, 826, 783, 698 cm^{-1}; ^1H NMR, (DMSO-d_6, δ ppm): 1.75 (1H, s, HC≡C), 4.1 (2H, s, ≡C–CH_2S), 7.25–7.30 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.30–7.35 (2H, m, C_3H and C_5H of phenyl), 7.6 (2H, d, C_3H and C_5H of pyridine), 8.57 (2H, d, C_2H and C_6H of pyridine). ^{13}C NMR, (DMSO-d_6, δ ppm): 22.12 (–CH_2–S), 72.78 (CH≡), 86.73 (≡C), 121.49 (2CH), 127.18 (CH), 130.36 (2CH), 130.59 (2CH), 133.58 (1CH), 133.99 (1CH), 150.25 (triazole C-3), 152.63 (2CH), 153.52 (triazole C-5). Anal. calcd. for $C_{16}H_{12}N_4S$: C, 65.73; H, 4.14; N, 19.16; S, 10.97. Found: C, 65.56; H, 3.92; N, 19.25; S, 11.09.

*3-[(Pent-2-yl)thio]-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole **15***

Yield 2.61 g, 77 %, m.p. 96–97 °C; IR (KBr) 3036, 2957, 1654, 1637, 836, 776, 694 cm^{-1}; ^1H NMR, ($CDCl_3$, δ ppm): 0.85 (3H, t, CH_3), 1.3–1.4 (2H, m, CH_2), 1.4–1.7 (5H, m, CH_3 and CH_2), 3.5–4.1 (1H, m, CH), 7.0–7.15 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.15–7.30 (2H, m, C_3H and C_5H of phenyl), 7.5 (2H, d, C_3H and C_5H of pyridine), 8.4 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{18}H_{20}N_4S$: C, 66.63; H, 6.21; N, 17.27; S, 9.88. Found: C, 66.81; H, 6.35; N, 16.98; S, 10.03.

*3-[(Pent-4-enyl)thio]-4-phenyl-5-pyridin-4-yl-2,4-dihydro-3H-1,2,4-triazole **16***

Yield 2.29 g, 73 %, m.p. 132–133 °C; IR (KBr) 3037, 2932, 1654, 1640, 991, 914, 837, 776, 700 cm^{-1}; ^1H NMR, ($CDCl_3$, δ ppm): 1.6–2.4 (4H, m, =CCH_2 CH_2), 4.7 (2H, d, =CH_2), 5.1 (1H, d, =CH), 7.0–7.15 (3H, m, C_2H, C_4H and C_6H of phenyl), 7.15–7.30 (2H, m, C_3H and C_5H of phenyl), 7.5 (2H, d, C_3H and C_5H of pyridine), 8.4 (2H, d, C_2H and C_6H of pyridine). Anal. calcd. for $C_{18}H_{18}N_4S$: C, 67.05; H, 5.63; N, 17.38; S, 9.94. Found: C, 66.92; H, 5.75; N, 17.45; S, 10.06.

In vivo carrageenan induced rat paw edema assay

Anti-inflammatory activity was determined by the carrageenan-induced rat paw edema method described by Winter et al. (Winter et al. 1962). Male Sprague–Dawley rats weighing 150–200 g (6–8 weeks old) were used in groups of six animals per group for the experiments. The animals were housed in a room with temperature of 22 ± 2 °C under a 12 h light/dark cycle. They were allowed free access to food and water ad libitum. The protocol for the animal experiments performed was approved by the Research Ethics Committee and Animal Care and Use Committee, Govt. of Saudi Arabia. Compounds were administered intravenously in dimethyl sulfoxide solution. Paw edema was induced by intradermal injection of 50 µL of 1 % λ-carrageenan (Sigma, USA) into the subplantar region of the right hind paw, after 1 h of compound administration. The paw volume was measured immediately after injection and after 2 h using a plethysmometer (UGO-Basile, Italy). The control group received only the vehicle. Increase in paw volume was compared with that in the control group and percent inhibition was calculated taking the values in the control group as 0 % inhibition.

In vitro COX inhibition assay

The final compounds were evaluated for their ability to inhibit ovine COX-1 and COX-2 enzymes [50 % percent inhibition is expressed in molar concentration (nmol)] (Sano et al. 2005). Inhibition of the enzymes was determined with the colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chemicals, USA) using ELISA reader.

Molecular docking

Molecular docking study was done using Surflex-Dock within Sybyl 8.1.1 on HPxw8400 workstation, linux_OS2x at laboratory of drug design, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan. The crystal structures of the two isozymes COX-1 with flurbiprofen (PDB entry code: 1cqe) (Picot et al. 1994) and the COX-2 with sc-558 (PDB entry code: 1cx2) (Kurumbail et al. 1996) were retrieved from the RCSB Protein Data Bank. Surflex-Dock uses an empirical scoring function and a patented search engine to dock ligands into a protein's binding site (Jain 2003). A Protomol, which was used to guide molecular docking, is a computational representation of the intended binding site to which putative ligands are aligned. Protomols can be produced by one of three routes: (Ruppert et al. 1997) (1) automatic: Surflex-Dock finds the largest cavity in the receptor protein; (2) ligand-based: a ligand in the same coordinate space as the receptor; (3) residue-based: specified residues in the receptor. Thus, a Protomol can be generated automatically or defined based on a cognate ligand or known active site. In the current paper, a Protomol was generated automatically. Two parameters determining the extent of the Protomol—a threshold parameter of 0.46 and a bloat parameter of 1 Å—were established. All the water molecules in 1cqe or 1cx2 (receptor) were deleted, and hydrogen atoms were added to them (Muthas et al. 2008; Clark 2008). The protein structure was utilized in subsequent docking experiments without energy minimization. In addition, treatment of docking small molecules (ligands) was as follows: preparation of 3D structures of ligands using Ligprep software module (Schrodinger, Inc., New York, NY). During this preparation step, hydrogen atoms were explicitly added, all possible ionization states were generated between pH 6.0 and pH 8.0 using the apic module, and the 3D molecular structures were minimized with OPLS 2005 force-field in Schrodinger software suite. Tautomers were also generated in this step. This preparation step was done on dell workstation under linux x86-32 at laboratory of drug design, School of pharmaceutical Sciences, Kitasato University, Tokyo, Japan. Charge calculation with energy minimization method: Powell; force field: tripos; charge: MMFF94; max iterations: 1,000; termination: 0.001 kcal/(mol*Å); root mean square (RMS) displacement: 0.001 Å; other parameters: treated by default. In the docking procedure, five additional starting conformations are used and ten binding poses per ligand were obtained, and the binding pose with the highest total score was taken into consideration for ligand–receptor interactions. Docking results were validated by finding the root mean squared deviation (RMSD) between the docking position calculated for sc-588 and flurbiprofen and that observed in their crystal structures.

Results and discussion

Chemistry

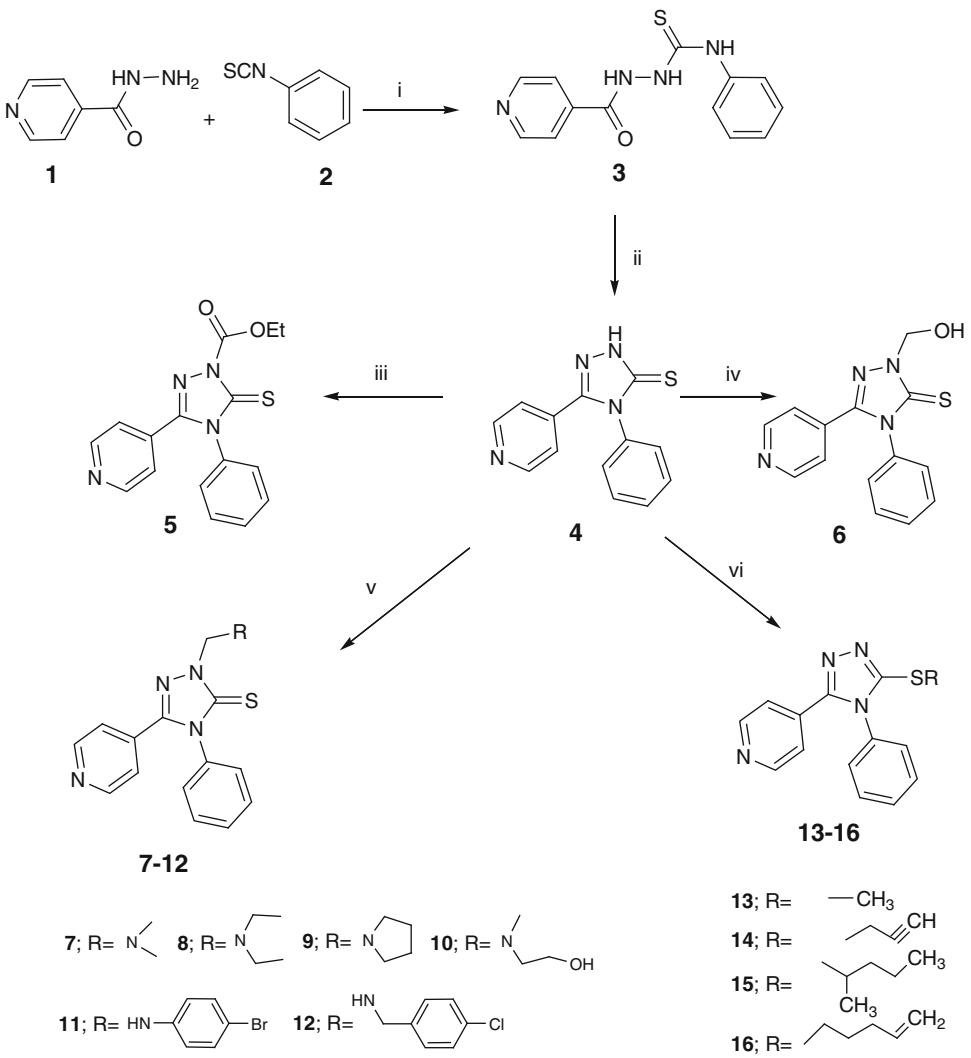
In Scheme 1, 1-isonicotinoyl-4-phenyl thiosemicarbazide (**3**) and 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-thiol (**4**) were prepared using a previously published procedure (Pomarnacka and Kornicka 2001; Bayrak et al. 2009; Vera et al. 2004). The reaction of 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-thiol (**4**) with ethyl chloroformate in presence of anhydrous sodium carbonate afforded compound **5**. The reaction of compound **4** with formaline solution resulted in 2-hydroxymethyl derivative (**6**). The reaction of 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-thiol (**4**) with the appropriate amine in the presence of formaldehyde solution afforded the corresponding Mannich base derivatives incorporating dimethylamine, diethylamine, pyrrolidine, *N*-methylethanolamine, *p*-bromoaniline, *p*-chlorobenzylamine (**7**–**12**). S-alkylation of 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-thiol (**4**) was performed by its reaction with several alkyl halides in basic media results in compounds (**13**–**16**) incorporating methyl, 2-pentyl, pent-4-enyl, 3-propynyl.

Anti-inflammatory activity

The triazoles (**5**–**16**) were screened for their anti-inflammatory activity using rat hind paw method of Winter et al. 1962 modified by Srimal and Dhawan 1974. Among the triazole series compounds **6**–**9** and **13**, **15** showed promising antiinflammatory activity compared with celecoxib as reference drug (Table 1). Compounds with aromatic or unsaturated moiety at position 2 or 3 respectively (**11**, **12**, **14** and **16**) are either moderately active. Compounds **5** and **10** showed no anti-inflammatory activity. In the literature it was shown that the triazole-scaffold containing compounds exhibit anti-inflammatory activity mediated through inhibition of COX-1 and 2 enzymes depending on the position and kind of substituent on the 1,2,4-triazole system (Navidpour et al. 2006). The compounds under study are speculated to show anti-inflammatory activity through COX-1 and/or COX-2.

In vitro cyclooxygenase inhibition

The effect of incorporation of substituent at position 2 and/or 3 of the central triazole ring on COX-2 selectivity and potency was determined by the IC50 values relative to the reference compound, celecoxib, using colorimetric COX (ovine) inhibitor screening assay (Table 1) (Kulmacz and Lands 1983). In vitro enzyme inhibition studies for compounds **5**–**16** showed variable COX-2 inhibitory activity. In general, for these compounds, COX-2 selectivity and potency were dependent upon steric properties of the substituent at position 2 and at position 3 on the central triazole



Scheme 1 General synthesis of compounds **5**–**16**. Reagents and conditions: *i* Ethanol, rt; *ii* NaOH; *iii* ethyl chloroformate, anhydrous sodium carbonate, DMF, reflux 7 h; *iv* formaline water, reflux 5 min; *v* amine, dimethyl formamide, formaldehyde, rt 3 h; *vi* alkyl halide, sodium ethoxide, ethanol, reflux 4 h

ring. Compounds (**6**–**9**, **13**, **15**) showed high in vitro potency and selectivity (**6**; COX-2 IC50 = 0.8 nM; COX-1 IC50 = 3.5 nM; S.I. = 4.38, **7**; COX-2 IC50 = 0.9 nM; COX-1 IC50 = 3.1 nM; S.I. = 3.44, **8**; COX-2 IC50 = 1.1 nM; COX-1 IC50 = 3.8 nM; S.I. = 3.46, **9**; COX-2 IC50 = 0.9 nM; COX-1 IC50 = 3.7 nM; S.I. = 4.11, **13**; COX-2 IC50 = 2.1 nM; COX-1 IC50 = 5.3 nM; S.I. = 2.52, **15**; COX-2 IC50 = 1.4 nM; COX-1 IC50 = 3.7 nM; S.I. = 2.64) relative to the reference drug celecoxib (COX-2 IC50 = 1.9 nM; COX-1 IC50 = 4.1 nM; S.I. = 2.16). Compounds **10**, **16** explored almost equal or slight lower potency and selectivity relative to the reference drug celecoxib (**10**; COX-2 IC50 = 2.1 nM; COX-1 IC50 = 4.5 nM; S.I. = 2.14, **16**; COX-2 IC50 = 2.6 nM; COX-1 IC50 = 4.4 nM; S.I. = 1.69). Compounds with ethoxycarbonyl (**5**) or aromatic (**11**, **12**) moiety at position 2 or alkyne (**14**) moiety at position 3 showed lower inhibition of COX-1 and COX-2 with lower selectivity value than celecoxib (**5**; COX-2 IC50 = 7.8 nM; COX-1 IC50 = 9.2 nM; S.I. = 1.18, **11**; COX-2 IC50 = 3.9 nM; COX-1 IC50 = 6.4 nM; S.I. = 1.64, **12**; COX-2 IC50 = 6.8 nM; COX-1 IC50 = 8.7 nM; S.I. = 1.38, **14**; COX-2 IC50 = 5.3 nM; COX-1 IC50 = 7.5 nM; S.I. = 1.42).

Molecular docking

COX-1 and 2 monomers each contain a 25-Å hydrophobic channel that originates at the membrane binding domain (MBD) and extends into the core of the globular domain (Picot et al. 1994; Kurumbail et al. 1996; Luong et al. 1996). The MBD forms the mouth and the first half of the channel. The NSAID binding site involves the upper half of this channel from Arg120 to near Tyr385. It is clear that the NSAID binding site corresponds to the cyclooxygenase active site (Rome and Lands 1975; Smith and DeWitt 1996). Several amino acids composing the upper half of the channel are uniquely important in cyclooxygenase catalysis. Twenty-four residues line the hydrophobic

Table 1 Percent inhibition of carrageenan paw edema (CPE) and COX-1 and COX-2 inhibitor activity of the compounds **5–16**

Compounds	AI activity		IC_{50} (nM)		Selectivity index (COX-1/COX-2)
	% inhibition at 1 h	% inhibition at 2 h	COX-1	COX-2	
5	0.0	5 ± 1.8	9.2	7.8	1.18
6	25 ± 1.8	66 ± 2.6	3.5	0.8	4.38
7	51 ± 1.5	76 ± 2.8	3.1	0.9	3.44
8	45 ± 1.1	66 ± 1.2	3.8	1.1	3.46
9	62 ± 3.4	66 ± 1.5	3.7	0.9	4.11
10	0	16 ± 5.9	4.5	2.1	2.14
11	12.5 ± 1.6	29 ± 3.8	6.4	3.9	1.64
12	16 ± 5.7	19 ± 6.3	8.7	6.8	1.38
13	46 ± 2.9	61 ± 4.1	5.3	2.1	2.52
14	10 ± 5.4	12 ± 4.9	7.5	5.3	1.42
15	36 ± 2.1	50 ± 2.5	3.7	1.4	2.64
16	9 ± 1.3	38 ± 1.8	4.4	2.6	1.69
Celecoxib	50 ± 2.1	71.5 ± 3.7	4.1	1.9	2.16

cyclooxygenase active site with only one difference between the isozymes—Ile at position 523 in COX-1 and Val at position 523 in COX-2. (Amino acids lining the hydrophobic cyclooxygenase active site channel include Leu117, Arg120, Phe205, Phe209, Val344, Ile345, Tyr348, Val349, Leu352, Ser353, Tyr355, Leu359, Phe381, Leu384, Tyr385, Trp387, Phe518, Ile/Val523, Gly526, Ala527, Ser530, Leu531, Gly533, Leu534.) Only three of the channel residues are polar (Arg120, Ser353, and Ser530). Ser530 is the site of acetylation by aspirin, (DeWitt et al. 1990; Lecomte et al. 1994; Loll et al. 1995) and Arg120 binds to the carboxylate groups of fatty acids and many NSAIDs (Greig et al. 1997; Kulmacz 1987; Mancini et al. 1995; Rouzer and Marnett 2003). The mechanism for a differential inhibition by classical and COX-2 NSAIDs can be rationalized to some extent based on differences between the cyclooxygenase active sites of COX-1 and 2. Substitution of Ile523 in COX-1 with Val523 in COX-2 results in the presence of a small side pocket adjacent to the active site channel, appreciably increasing the volume of the COX-2 active site (Luong et al. 1996). This change is compounded by the substitution of Ile434 in COX-1 with Val434 in COX-2, within the second shell of amino acids surrounding the cyclooxygenase active site. The Ile to Val substitution at position 434 outside the COX-2 catalytic centre further increases the effective size of the active site channel by enhancing the local mobility of side chains within the side pocket. The combination of these two differences at positions 523 and 434 in COX-2 causes a movement of Phe518 that further increases the size of the side pocket. The larger main channel combined with the extra nook increases the volume of the COX-2 NSAID binding site by about 20 % over that in COX-1 (Luong et al. 1996). This extra size is a structural feature exploited by COX-2 inhibitors. Finally, the substitution of His513 in COX-1 with Arg513 in COX-2 results in a stable positive charge being placed at the centre of this pocket, which can interact with polar moieties entering the pocket (Kurumbail et al. 1996). For example, Arg513 appears to interact with the 4-methysulfonyl or 4-sulfonamoylphenyl substituents of diaryl heterocyclic COX-2 inhibitors.

Docking studies of the synthesized compounds **5–16** in the active sites of both COX-1 (PDB code: 1CQE) (Picot et al. 1994) and COX-2 (PDB code: 1CX2) (Kurumbail et al. 1996) were performed in order to get further insight into the nature of interactions between the compounds and the active site amino acids to rationalize the obtained biological results. For validation of our docking procedure

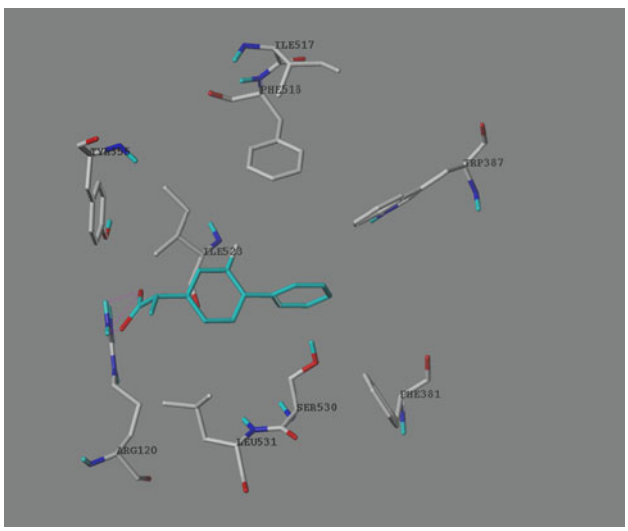


Fig. 1 COX-1: the docked co-crystallized flurobiprofen (from 1CQE.pdb, *coloured cyan*). Hydrogen bonds are displayed in *magenta*

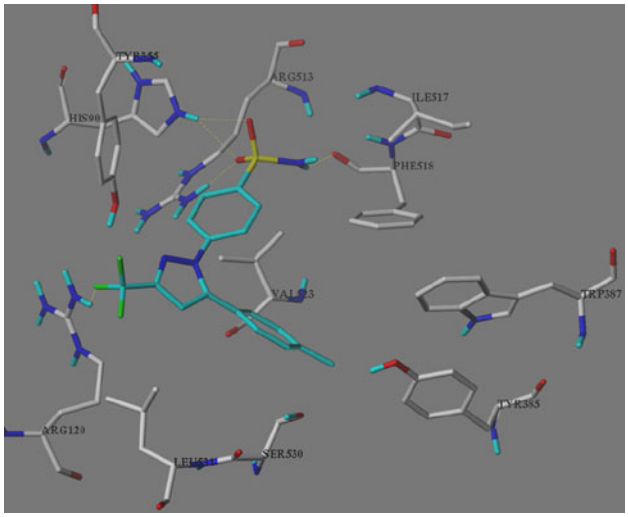


Fig. 2 COX-2: the docked co-crystallized SC-558 (from 1CX2.pdb, *coloured cyan*). Hydrogen bonds are displayed in *yellow*

Table 2 Calculated docking score for COX-1 and COX-2

Compounds	Total docking score	
	COX-1	COX-2
5	5.42	7.87
6	4.59	6.63
7	5.56	8.60
8	6.21	9.37
9	4.89	8.41
10	6.61	8.10
11	4.25	4.64
12	4.31	5.74
13	5.63	6.51
14	6.13	6.31
15	7.56	8.36
16	7.23	9.15
Celecoxib	4.31	7.99
Flurbiprofen	6.8	6.19

flurbiprofen and sc-558 were docked inside 1CQE and 1CX2 respectively. The docking results of 1CQE and 1CX2 were able to reproduce the experimentally observed interaction mode (Limongell et al. 2010) with rmsd values between experimentally used and docked ligand structure of 0.31 and 0.42 respectively (Figs. 1, 2). Table 2 shows that compounds **6–10** and **13**, **15**, **16** explored comparable interactions, with high docking score at COX-1 and COX-2, with that of the co-crystallized flurbiprofen and sc-558 while compounds **11**, **12**, **14** explored lower docking score value than that of flurbiprofen and sc-558. Docking score values of compounds **6–16** were found in agreement with their in vitro screening results against the enzymes. While compound **5** showed similar binding mode and hydrogen bonding with high docking score at COX-1 and COX-2 as well as flurbiprofen and sc-558. This was not in agreement with its lower in vitro activity against both COX-1 and COX-2.

Whether this binding results or not into a functional inhibition is more complicated to predict since it may be related to kinetic of dissociation rather than to the stability of the complex. The failure of static docking experiments in predicting the inactivity of analogues, closely related to COX-2 active diaryl heterocyclic derivatives can be commented on the light of a recent paper by Limongell et al. 2010 who put forward not only the importance of the stability of the binding mode, but also the importance of the dynamic path of ligand(s) to reach the binding pose. In this context, Limongell et al. 2010 proposed the existence of alternative binding modes of diaryl heterocyclic derivatives to COX-2. It can be speculated that the reason for the inactivity of some compounds may reside in their inability to achieve one or more alternative binding modes possibly relevant to functional inhibition (Limongell et al. 2010).

As examples the docking solution obtained for compounds **9** and **15** at COX-2 are shown in Figs. 3 and 4. The docked compounds fit well into the binding pocket and show hydrogen bonds with Arg513. The pyridine moiety at the C-5 position of the triazole ring fits into the COX-2 secondary pocket and makes hydrogen bond between its ring-nitrogen and Arg513, showing a similar interaction as the sulfonamide group of SC-588. The phenyl ring at C-4 position of the triazole ring occupies the same position of

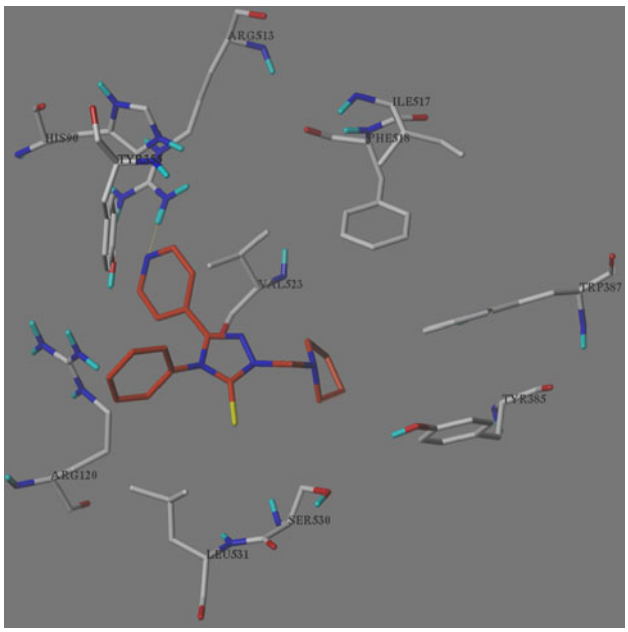


Fig. 3 COX-2: the docked compound **9** (*coloured orange red*). Hydrogen bond is displayed in *yellow*

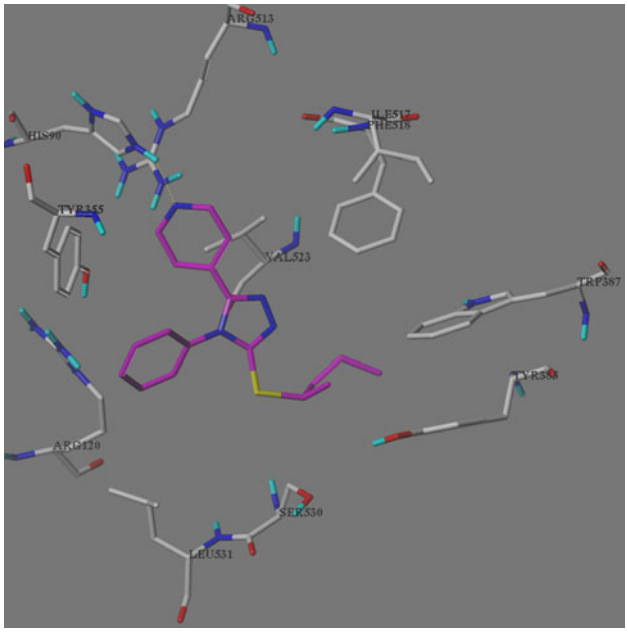


Fig. 4 COX-2: the docked compound **15** (*coloured magenta*). Hydrogen bond is displayed in *yellow*

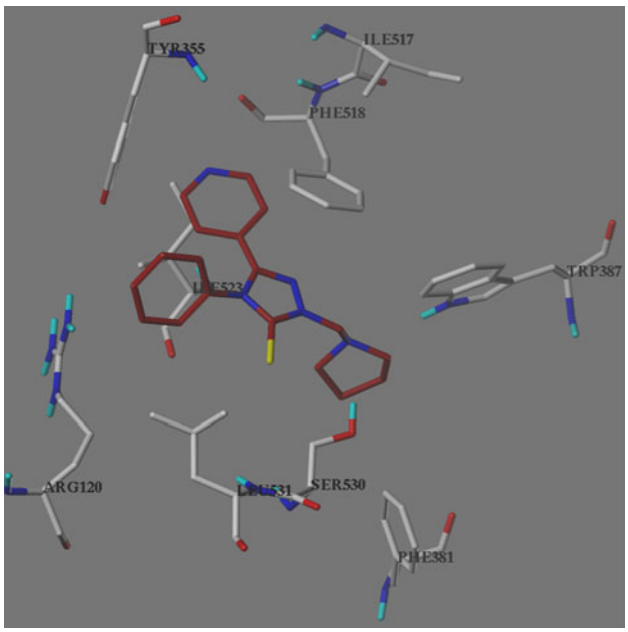


Fig. 5 COX-1: the docked compound **9** (*coloured orange red*)

trifluoromethyl of sc-558, the N-2 or C-3 substituent could occupy the same position of the the bromophenyl ring of sc-558. As examples the docking solution obtained for compounds **9** and **15** at COX-1 are shown in Figs. 5 and 6. The docked compounds fitted well into the binding pocket while did not show hydrogen bonding with Arg120. The central triazole ring adopts the same position as the central aromatic ring of flurbiprofen. The S-pent-2-yl or

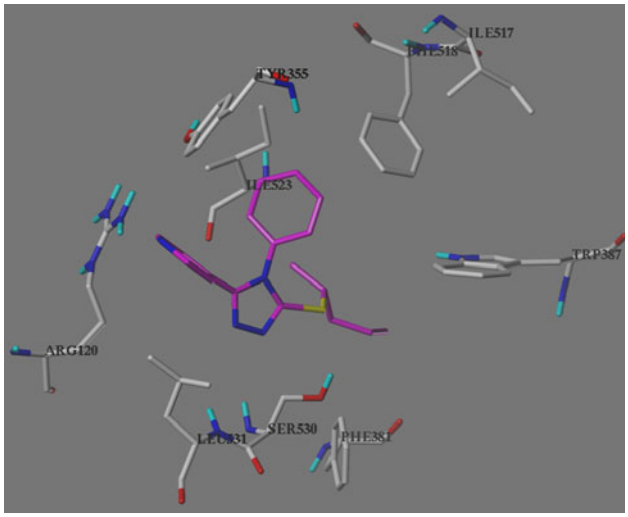


Fig. 6 COX-1: the docked compound **15** (*coloured magenta*)

pyrrolidinylmethyl substituent occupied the same position of the terminal aromatic ring of flurbiprofen.

Conclusions

In summary, a series of triazole compounds were synthesized and biologically evaluated. Some of the synthesized derivatives were more potent and more selective on COX-2, compared to celecoxib. In animal model, compound **7** was slightly more active and compounds **6**, **8**, **9** were slightly less active than celecoxib, reference drug. However, compounds **5** and **10** almost were biologically inactive in animal model. Compounds **6–9** and **15** explored higher anti-COX-2 activity and selectivity than that of celecoxib. Compounds **10** and **13** explored same anti-COX potency and COX-2 selectivity, as well as celecoxib. Compounds **5**, **11**, **12** and **14** showed lower anti-COX activity with lower COX-2 selectivity.

Molecular docking was further performed to study the inhibitor-COX protein interactions. After analysis of the binding model of compounds **9** and **15** with COX-2, it was found that these compounds occupied same orientation as well as sc-558 and showed hydrogen with Arg513 in COX-2 binding site. The analysis of their binding model with COX-1, it was found that these compounds fitted well to the same binding sites of flurbiprofen with an additional hydrophobic interaction with Ile523 while did not show any hydrogen interaction. Among these compounds, it could be concluded that compound **9** had been demonstrated to show significant selective COX-2 inhibitory activity as a potential antiinflammatory agent. The result of this work might be helpful for the design and synthesis of more selective COX-2 inhibitors with stronger activity.

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References

AbdelMoallem, S., F. Hadizadeh, and I. Yavar. 2011. New *N*-aryl-4-(methysulfony)aminobenzenesulfonamides as selective COX-2 inhibitors. *Journal of Biological Sciences* 11: 496–499.

Ali, A.M., G.E. Saber, N.M. Mahfouz, M.A. EI-Gendy, A.A. Radwan, and M.A. Hamid. 2007. Synthesis and three-dimensional qualitative structure selectivity relationship of 3,5-disubstituted-2,4-thiazolidinedione derivatives as COX-2 inhibitors. *Archives of Pharmacal Research* 30: 1186–1204.

Bayrak, H., A. Demirbas, S.A. Karaoglu, and N. Demirbas. 2009. Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities. *European Journal of Medicinal Chemistry* 44: 1057–1066.

Catella-Lawson, F., and L.J. Crofford. 2001. Cyclooxygenase inhibition and thrombogenicity. *American Journal of Medicine* 110: 28S–32S.

Clark, R.D. 2008. A ligand's-eye view of protein binding. *Journal of Computer-Aided Molecular Design* 22: 507–521.

Dannhardt, G., and S. Laufer. 2000. Structural approaches to explain the selectivity of COX-2 inhibitors: is there a common pharmacophore? *Current Medicinal Chemistry* 7: 1101–1112.

DeGaetano, G., M.B. Donati, and C. Cerletti. 2003. Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors. *Trends in Pharmacological Sciences* 24: 245–252.

DeWitt, D.L., E.A. el-Harith, S.A. Kraemer, M.J. Andrews, E.F. Yao, R.L. Armstrong, and W.L. Smith. 1990. The aspirin and heme-binding sites of ovine and murine prostaglandin endoperoxide synthases. *Journal of Biological Chemistry* 265: 5192–5198.

Greig, G.M., D.A. Francis, J.P. Falgueyret, M. Ouellet, M.D. Percival, P. Roy, C. Bayly, J.A. Mancini, and G.P. O'neill. 1997. The interaction of arginine 106 of human prostaglandin G/H synthase-2 with inhibitors is not a universal component of inhibition mediated by nonsteroidal anti-inflammatory drugs. *Molecular Pharmacology* 52: 829–838.

Jain, A.N. 2003. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *Journal of Medicinal Chemistry* 46: 499–511.

Kulmacz, R.J., and W.E.M. Lands. 1983. Requirements for hydroperoxide by the cyclooxygenase and peroxidase activities of prostaglandin H synthase. *Prostaglandins* 25: 531–540.

Kulmacz, R.J. 1987. Prostaglandin G2 levels during reaction of prostaglandin H synthase with arachidonic acid. *Prostaglandins* 34: 225–240.

Kurumbail, R.G., A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, and W.C. Stallings. 1996. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* 384: 644–648.

Labanauskas, L., V. Kalcas, E. Udrenaite, P. Gaidelis, A. Brukstus, and U. Dauksas. 2001. Synthesis of 3-(3,4-dimethoxyphenyl)-1H-1,2,4-triazole-5-thiol and 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole derivatives exhibiting anti-inflammatory activity. *Pharmazie* 56: 617–619.

Lecomte, M., O. Laneuville, C. Ji, D.L. DeWitt, and W.L. Smith. 1994. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. *Journal of Biological Chemistry* 269: 13207–13215.

Limongell, V., M. Bonomi, L. Marinelli, F.L. Gervasio, A. Cavalli, E. Vovellino, and M. Parrinello. 2010. Molecular basis of cyclooxygenase enzymes (COXs) selective inhibition. *PNAS* 107: 5411–5416.

Loll, P.J., D. Picot, and R.M. Garavito. 1995. The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase. *Natural Structural Biology* 2: 637–643.

Luong, C., A. Miller, J. Barnett, J. Chow, C. Ramesha, and M.F. Browner. 1996. Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Natural Structural Biology* 3: 927–933.

Mancini, J.A., D. Riendeau, J.P. Falgueyret, P.J. Vickers, and G.P. O'Neill. 1995. Arginine 120 of prostaglandin G/H synthase-1 is required for the inhibition by nonsteroidal anti-inflammatory drugs containing a carboxylic acid moiety. *Journal of Biological Chemistry* 270: 29372–29377.

Meade, E.A., W.L. Smith, and D.L. DeWitt. 1993. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *Journal of Biological Chemistry* 268: 6610–6614.

Moreau, A., P.N.P. Rao, and E.E. Knaus. 2006. Synthesis and biological evaluation of acyclic triaryl (*Z*)-olefins possessing a 3,5-di-*tert*-butyl-4-hydroxyphenyl pharmacophore: dual inhibitors of cyclooxygenases and lipoxygenases. *Bioorganic & Medicinal Chemistry* 14: 5340–5350.

Mukherjee, D., S.E. Nissen, and E.J. Topol. 2001. Risk of cardiovascular events associated with selective COX-2 inhibitors. *JAMA* 286: 954–959.

Muthas, D., Y.A. Sabnis, M. Lundborg, and A. Karlén. 2008. Is it possible to increase hit rates in structure-based virtual screening by pharmacophore filtering? An investigation of the advantages and pitfalls of post-filtering. *Journal of Molecular Graphics and Modelling* 26: 1237–1251.

Navidpour, L., H. Shafaroodi, K. Abdi, M. Amini, M.H. Ghahremani, A.R. Dehpour, and A. Shafiee. 2006. Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4H-1,2,4-triazoles as selective COX-2 inhibitors. *Bioorganic & Medicinal Chemistry* 14: 2507–2517.

Penning, T.D., J.J. Tally, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, D.J. Rogers, S.S. Rogier, G.D. Yu, E.G. Anderson, J.N. Burton, S.A. Cogburn, C.M. Gregory, R.S. Koboldt, W.E. Perkins, K. Seibert, A.W. Veenhuizen, Y.Y. Zhang, and P.C. Isakson. 1997. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). *Journal of Medicinal Chemistry* 40: 1347–1365.

Picot, D., P.J. Loll, and R.M. Garavito. 1994. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature* 367: 243–249.

Plount-Price, M.L., and W.L. Jorgensen. 2000. Analysis of binding affinities for celecoxib analogues with COX-1 and COX-2 from combined docking and Monte Carlo simulations and insight into the COX-2/COX-1 selectivity. *Journal of the American Chemical Society* 122: 9455–9466.

Pomarnacka, E., and A. Kornicka. 2001. Synthesis and in vitro anticancer and anti-HIV evaluation of new 2-mercaptobenzenesulfonamides. *Il Farmaco* 56: 571–577.

Praveen, R.P.N., A. Mohsen, L. Huiying, G.H. Amgad, and E.K. Edward. 2003. Design, synthesis and biological evaluation of 6-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: a novel class of diarylheterocyclic selective cyclooxygenase-2 inhibitors. *Journal of Medicinal Chemistry* 46: 4872–4882.

Ranatunge, R.R., R.A. Earl, D.S. Garvey, D.R. Janero, L.G. Letts, A.M. Martino, M.G. Murty, S.K. Richardson, D.J. Schwalb, D.V. Young, and I.S. Zemtseva. 2004. 3-(2-Methoxytetrahydrofuran-2-yl)pyrazoles: a novel class of potent, selective cyclooxygenase-2 (COX-2) inhibitors. *Bioorganic & Medicinal Chemistry Letters* 14: 6049–6052.

Riendeau, D., M.D. Percival, C. Brideau, C.S. Dube, D. Ethier, J.P. Falgueyret, R.W. Friesen, R. Gordon, G. Greig, J. Guay, Y. Girard, P. Prasit, R. Zamboni, I.W. Rodger, M. Gresser, A.W. Ford-Hutchinson, R.N. Young, and C.C. Chan. 2001. Etoricoxib (MK-0663): preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. *Journal of Pharmacology and Experimental Therapeutics* 296: 558–566.

Rome, L.H., and W.E. Lands. 1975. Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. *Proceedings of the National Academy of Sciences of the United States of America* 72: 4863–4865.

Rouzer, C.A., and L.J. Marnett. 2003. Mechanism of free radical oxygenation of polyunsaturated fatty acids by cyclooxygenases. *Chemical Reviews* 103: 2239–2304.

Ruppert, J., W. Welch, and A.N. Jain. 1997. Automatic identification and representation of protein binding sites for molecular docking. *Protein Science* 6: 524–533.

Sano, H., T. Noguchi, A. Tanatani, Y. Hashimoto, and H. Miyachi. 2005. Design and synthesis of subtype-selective cyclooxygenase (COX) inhibitors derived from thalidomide. *Bioorganic & Medicinal Chemistry* 13: 3079–3091.

Silverstein, F.E., G. Faich, J.L. Goldstein, L.S. Simon, T. Pincus, A. Whelton, R. Makuch, G. Eisen, N.M. Agrawal, W.F. Stenson, A.M. Burr, W.W. Zhao, J.D. Kent, J.B. Lefkowith, K.M. Verburg, and G.S. Geis. 2000. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS Study: a randomized controlled trial. *JAMA* 284: 1247–1255.

Smith, W.L., and D.L. DeWitt. 1996. Prostaglandin endoperoxide H synthases-1 and -2. *Advances in Immunology* 62: 167–215.

Srimal, R.C., and B.N. Dhawan. 1974. Biological, toxicological and clinical evaluation. *Indian Journal of Pharmacology* 3: 4–13.

Sui, Z., J. Guan, M.P. Ferro, K. McCoy, M.P. Wachter, W.W. Murray, M. Singer, M. Steber, D.M. Ritchie, and D.C. Argentieri. 2000. 1,3-Diarylcycloalkanopyrazoles and diphenyl hydrazides as selective inhibitors of cyclooxygenase-2. *Bioorganic & Medicinal Chemistry Letters* 10: 601–614.

SYBYL Molecular Modelling Software. 2006. Tripos Inc., St. Louis, http://www.tripos.com. Accessed July 2010.

Talley, J.J., D.L. Brown, J.S. Carter, M.J. Graneto, C.M. Koboldt, J.L. Masferrer, W.E. Perkins, R.S. Rogers, A.F. Shaffer, Y.Y. Zhang, B.S. Zweifel, and K. Seibert. 2000. 4-[5-Methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2. *Journal of Medicinal Chemistry* 43: 775–777.

Tanaka, Y., S.L. xWard, and W.L. Smith. 1987. Immunohistochemical and kinetic evidence for two different prostaglandin H-prostaglandin E isomerases in sheep vesicular gland microsomes. *Journal of Biological Chemistry* 262: 1374–1381.

Tozkoparan, B., E. Kupeli, E. Yesilada, S. Isik, M. Ozalp, and M. Ertan. 2005. Synthesis and evaluation of analgesic/anti-inflammatory and antimicrobial activities of 3-substituted-1,2,4-triazole-5-thiones. *Arzneimittelforschung* 55: 533–540.

Vera, K., Z. Lenka, W. Karel, K. Jamila, and M. Ute. 2004. Synthesis and antimycobacterial activity of 1,2,4-triazole 3-benzylsulfanyl derivatives. *Il Farmaco* 59: 279–288.

Winter, C.A., E.A. Risley, and G.W. Nuss. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine* 111: 544–547.

Wolfe, M.M., D.R. Lichtenstein, and G. Singh. 1999. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *New England Journal of Medicine* 340: 1888–1899.

Wujec, M., and P. Paneth. 2008. Mechanism of 4-methyl-1,2,4-triazol-3-thione reaction with formaldehyde. *Journal of Physical Organic Chemistry* 21: 345–348.

Wujec, M., M. Pitucha, M. Dobosz, U. Kosikowska, and A. Malm. 2003. Synthesis and biological action of 1-substituted-3-R-4-phenyl-delta 2–1,2,4-triazoline-5-thion. *Acta Poloniae Pharmaceutica* 60: 451–456.

Zarghi, A., F.S. Javid, R. Ghodsi, O.G. Dadrass, B. Daraei, and M. Hedayati. 2011. Design, synthesis and biological evaluation of new 5,5-diarylhydantoin derivatives as selective cyclooxygenase-2 inhibitors. *Scientia Pharmaceutica* 79: 449–460.