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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 979–982

Synthesis and evaluation of *S*-4-(3-thienyl)phenyl-α-methylacetic acid

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Received 7 August 2003; revised 24 November 2003; accepted 26 November 2003

Abstract—Herein we report an efficient procedure to synthesize *S*-4-(3-thienyl)phenyl-α-methylacetic acid, an enantiomerically pure intermediate of a recently approved nonsteroidal antiinflammatory cyclooxygenase inhibitor, atliprofen [methyl *RS*-4-(3-thienyl)-phenyl-α-methylacetate]. The interactions of the active *S*-isomer of the acid were theoretically compared with those of *S*-ibuprofen through molecular docking studies using COX-1 and COX-2 protein structures. The results were corroborated by in vitro and in vivo studies.

Prostaglandins are important biological mediators of inflammation, originating from biotransformation of arachidonic acid catalyzed by cyclooxygenase.[1] In the early 1990s, it was discovered that the enzyme exists as two isoforms, one constitutive (COX-1) and the other inducible (COX-2).[2] COX-1 is an enzyme that is constitutively expressed in humans in many tissues, including those of the gastrointestinal (GI) tract, platelets, kidneys, and brain.[3] It is involved in several physiological functions, including the generation of prostaglandins, which protect the mucosal integrity of the GI tract, maintain renal blood flow, and promote platelet aggregation. Conventional NSAIDs inhibit COX-1 and thus can have adverse effects on gastrointestinal tract. COX-2 is primarily an inducible enzyme that is not found in most tissues under normal conditions but is produced in response to pain and inflammation. Low basal concentrations of COX-2 would be expected to produce anti-inflammatory effects without causing the adverse effects associated with COX-1 inhibitors.[3] Enormous resources have, therefore, been invested in developing selective COX-2 inhibitors based on the hypothesis that these compounds will reduce pain, fever and inflammation without causing gastrointestinal injury.[4] However, for several reasons, including increased cardiovascular risks, acute renal failure, its expression in healthy stomach mucosa, role in defensive response of the stomach on mild irritation, the use of selective COX-2 inhibitors is under debate.[5] The most widely used class of NSAIDs in the current therapy of inflammatory conditions still remains the 2-arylpropionic acids or 'profens' which are nonselective and inhibit both cyclooxygenases.[6] Various drugs belonging to this class are naproxen, ibuprofen, ketoprofen, flurbiprofen.

Currently, a large number of COX inhibitors are available in the market. However, the gastrointestinal irritation continues to be among the most severe side effects thereby restricting their wide applications for therapeutic uses. IDPH-8261 [**1a**, methyl 4-(3-thienyl)phenyl-α-methylacetate][7] is a derivative of arylpropionic acid with remarkable safety profile and has been

Keywords: Arylpropionic acid; Docking; Cyclooxygenase.
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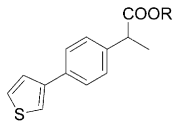
doi:10.1016/j.bmcl.2003.11.066

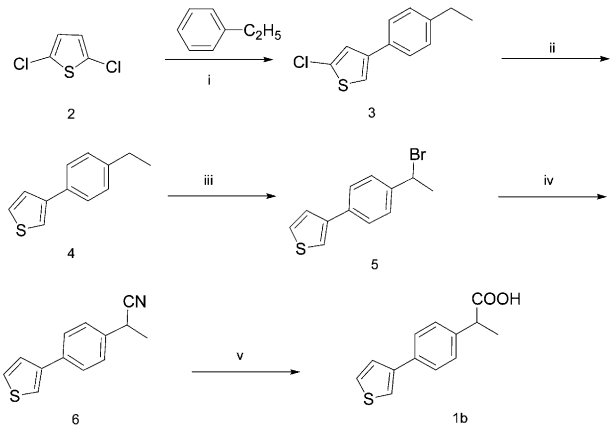


1a. R = CH_3
1b. R = H

Figure 1. Atliprofen (**1a**) and 4-(3-thienyl)phenyl-α-methylacetic acid (**1b**).

approved by WHO under international non-proprietary name (INN) atliprofen. This compound has completed all the three pre-marketing phases of clinical trials and is a potential NSAID with analgesic, antiinflammatory and antiarthritic properties.[8] Herein we report an improved synthesis of racemic 4-(3-thienyl)phenyl-α-methylacetic acid (**1b**),[9] an intermediate of atliprofen. Since antiinflammatory activity of these profens is mainly due to *S*-enantiomer,[10] *S*-4-(3-thienyl)phenyl-α-methylacetic acid was prepared and its activity was compared with that of ibuprofen, through molecular modelling, in vitro and in vivo studies.

The synthesis of atliprofen has been reported starting with *p*-tolylaldehyde.[11–13] The reported procedure is lengthy involving 11 steps which necessitates usage of a large number of reagents and provides **1b** in an overall yield of 16.95%, making it highly uneconomical. So, a new and more efficient process (Scheme 1) was developed which provided **1b** in five simple steps, from 2,5-dichlorothiophene (**2**), in an overall yield of 25.5% (Fig. 1). Electrophilic substitution of **2** with ethylbenzene furnished 4-[(4-ethyl)phenyl]-2-chlorothiophene (**3**) which was dechlorinated to get 3-[(4-ethyl)phenyl]thiophene (**4**).[14] Further, benzylic bromination using *N*-bromosuccinimide in the presence of a free radical initiator yielded 4-(3-thienyl)-α-methylbenzylbromide (**5**) which was converted to the corresponding acetonitrile using an alkali metal cyanide. The hydrolysis of 4-(3-thienyl)phenyl-α-methylacetonitrile (**6**) yielded **1b**. The spectral data of the synthesized compounds are provided.[15] The synthesized racemic acid (**1b**) was resolved to give *S*-4-(3-thienyl)phenyl-α-methylacetic



Scheme 1. Reagents and conditions: (i) $AlCl_3$, dry DCM, 0 to rt, 1.5 h, 62%; (ii) H_2, 10% Pd/C, rt, 72 h, 96%; (iii) NBS, benzoylperoxide, CCl_4, reflux, 2 h, 99%; (iv) NaCN, dry DMF, rt, 12 h, 60%; (v) HCl, CH_3COOH, reflux, 6 h, 72%.

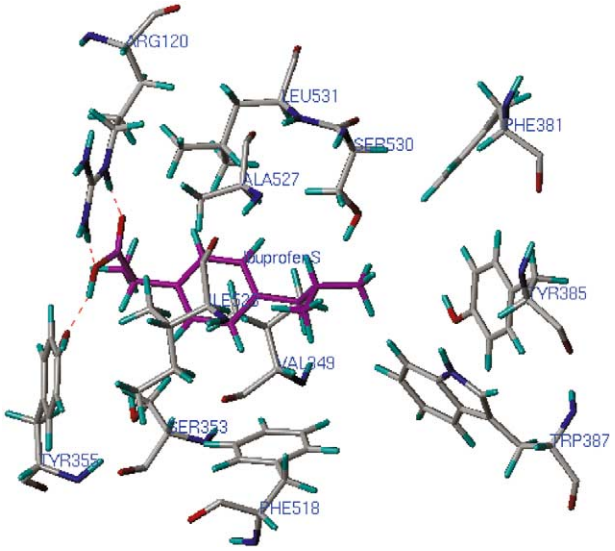


Figure 2. *S*-Ibuprofen docked in COX-1.

acid (*S*-**1b**, *ee* 95%) by fractional crystallization of its diastereomeric salt formed with *N*-methyl-D-glucamine[16] from anhydrous ethanol. The comparison of *S*-**1b** with *S*-ibuprofen was carried out via computational and biological studies.

All the computational work was carried out on an SGI-Octane workstation using Sybyl6.8[17] suite of molecular modelling software. The FlexX program[17b] was used to perform the molecular docking of **1b** in the active site of COX-1 and COX-2 (Figs. 2–5). 1EQG (PDB code) is a complex of ibuprofen with the protein COX-1 and 3PGH is a complex of flurbiprofen with the protein COX-2 in the protein data bank.[18] These were used as the standard crystal structures for docking **1b** in COX-1 and COX-2 respectively. The active site was defined as the spherical cavity of 7.0 Å surrounding the inhibitor in the COX crystal structure. FlexX scores were obtained for 50 conformers of each ligand. All the 50 conformers of each of the two ligands were superimposed on the standard crystal structure and the best conformers were considered for further analysis (Tables 1 and 2). Further, the best conformer was merged into the active site of the crystal structure, the hydrogen atoms were added to the crystal structure and then they were optimized using molecular mechanics methods. Each structure was subjected to molecular dynamics simulation at 300 K for 2000 fs with 1 fs step size and

Table 1. Analysis of docking in COX-1 enzyme

Ligand	FlexX score	I.E. (kcal/mol)	S.E. (kcal/mol)
S-Ibuprofen	−15.58	−50.963	4.89
S-**1b**	−16.76	−52.03	6.9

Table 2. Analysis of docking in COX-2 enzyme

Ligand	FlexX score	I.E. (kcal/mol)	S.E. (kcal/mol)
S-Ibuprofen	−14.46	−42.341	6.246
S-**1b**	−12.17	−38.606	1.254

Table 3. IC_{50} values of NSAIDs for COX-1 and COX-2 enzymes

Drug	IC_{50} (μM)		COX-2/COX-1
	COX-1	COX-2	
S-Ibuprofen	2.26	265.5	117.5
S-**1b**	45.24	179.7	3.97

Table 4. ED_{50} of the drugs used in study

Compd	ED_{50} (mg/kg)
S-**1b**	1.85±0.26
1b	5.74±1.62
S-Ibuprofen	7.08±1.97
RS-Ibuprofen	33.22±2.05
S-Naproxen	0.99±0.28
RS-Naproxen	2.35±0.36

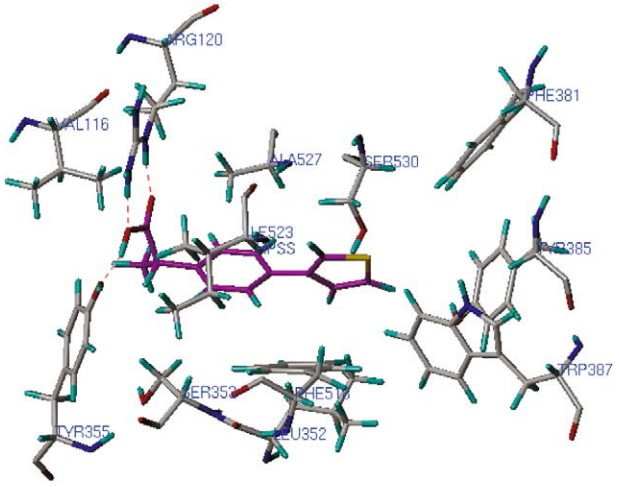


Figure 3. *S*-4-(3-Thienyl)phenyl-α-methylacetic acid docked in COX-1.

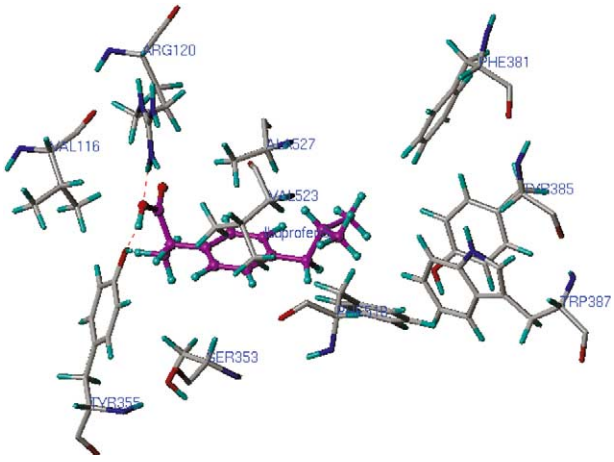


Figure 4. *S*-Ibuprofen docked in COX-2.

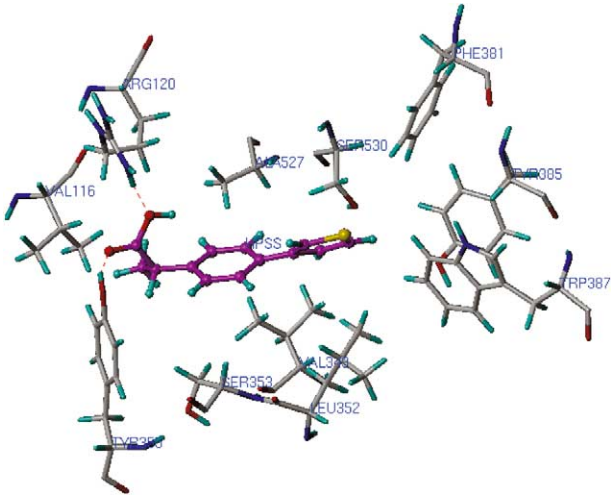


Figure 5. *S*-4-(3-Thienyl)phenyl-α-methylacetic acid docked in COX-2.

values were recorded at every 10 fs. The average structure of the dynamics (200 structures) was taken and minimized by keeping backbone as aggregate. The minimization was done using 1000 steps of the gradient method using MMFF94 as force field and MMFF94 charges. The interaction and strain energies were calculated as given below:

$$\text{Interaction Energy (I.E.)} = E_{\text{complex}} - [E_{\text{ligand}} + E_{\text{protein}}]$$

(all three in docked orientations).

$$\text{Strain Energy (S.E.)} = \text{E of ligand in docked orientation} - \text{E of minimized ligand}$$

While docking in COX-1 the carboxylate group forms two salt bridges with the guanidinium group of Arg120 and a hydrogen bond with the phenolic hydroxyl group of Tyr355. From Table 1, it is observed that, when Flex-X score and interaction energies are taken into account, *S*-**1b** has slightly greater affinity for the COX-1 than ibuprofen. But according to strain energy *S*-ibuprofen has more affinity for COX-1 as compared to *S*-**1b**. While docking in COX-2 the carboxylate group forms a salt bridge with the guanidinium group of Arg120 and a hydrogen bond with the phenolic hydroxyl group of Tyr355. From Table 2 it is observed that the FlexX score and interaction energy of *S*-ibuprofen was less for COX-2 as compared to those of *S*-**1b** but the strain energy was very high. Thus, *S*-**1b** has more affinity for COX-2 when compared with *S*-ibuprofen according to the strain energy. The binding site of COX-2 is slightly larger than that of COX-1 due to the presence of bulkier side chain of Ile523 in COX-1 versus Val523 in COX-2.[19] This is also reflected in case of *S*-**1b** which has strain energy of 6.9 in COX-1 whereas 1.25 in COX-2. In case of ibuprofen, isobutyl group is relatively small and more flexible compared to thienyl ring of **1b**, so it can adjust in COX-1 active site as well and the difference in strain energy is only 1.3 kcal/mol compared to *S*-**1b** where the difference is 5.7 kcal/mol. These results indicate that, based on the strain energy, the binding of *S*-**1b** is more favoured in the COX-2 active site rather than in COX-1 where it is relatively more strained. Binding of *S*-ibuprofen, on the other hand, is equally favoured in both COXs. Further biological evaluation showed that strain

energy obtained through docking studies correlates with in vitro and in vivo studies.

A radiochemical enzyme assay was carried out for assessing antiinflammatory activity in terms of COX-1 and COX-2 catalyzed prostaglandin biosynthesis in vitro. *S*-ibuprofen and *S*-**1b** were tested for cyclooxygenase enzyme inhibition activity. The relative activities of the two compounds were established by comparing their IC_{50} values with those of ibuprofen in the same model.[20] The in vitro study was done on purified enzymes, the source for COX-1 being ram seminal vesicle and that for COX-2 being sheep placenta. IC_{50} values obtained are shown in Table 3. The results from in vitro studies indicate that *S*-**1b** is more selective for COX-2 enzyme (about 1.47 times more) as compared to *S*-ibuprofen and is 20 times less effective on COX-1, which indicates that it will have less adverse effects like gastric damage as compared to *S*-ibuprofen. The stereoisomer *S*-**1b** was evaluated for its antiinflammatory activity in carrageenan-induced hind paw edema in a, rat model.[21] Male Sprague–Dawley rats of 8–10 weeks age weighing 220–250 g were used in the study. The drug was administered orally as a suspension in 1% CMC. Acute hind paw edema was produced by injecting 0.1 mL of carrageenan (prepared as 1% solution in sterile normal saline) subcutaneously to the plantar surface of the right hind paw of the rats half an hour after injecting the drug. The rat paw volume up to the ankle joint was measured using plethysmometer at 60, 120 and 180 min post carrageenan injection. ED_{50} of the drugs was calculated by linear regression of the response with various dose (0.3–100 mg/kg) at 180 min. Based on ED_{50} values (Table 4), the order of potency was:

S-naproxen > *S*-**1b** ≈ *S*-naproxen > **1b** ≈ *S*-ibuprofen >> *RS*-ibuprofen.

In conclusion, the synthesis of 4-(3-thienyl)phenyl-α-methylacetic acid was accomplished through a more efficient process. This racemic acid was resolved to *S*-4-(3-thienyl)phenyl-α-methylacetic acid through fractional crystallization using *N*-methyl-D–glucamine as a chiral base. The *S*-isomer was tested for its biological activity and was found to have better pharmacological profile as compared to *S*-ibuprofen. The in vitro and in vivo results were rationalized through docking studies on cyclooxygenase enzymes, both COX-1 and COX-2 using FlexX program followed by molecular dynamics.

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15. ^{1}H NMR ($CDCl_3$), IR, MS (EI), mp for representative compounds: **3**: ^{1}H NMR ($CDCl_3$): δ 1.25 (3H, t, J = 7.5), 2.66 (2H, q, J = 7.5), 7.13–7.24 (4H, m), 7.42 (2H, d, J = 8.1); IR (KBr): 3100, 2965, 1501, 1425, 1008, 822, 740 cm^{-1}; MS (*m*/*z*): 222 (M^+); mp: 97 °C. **4**: ^{1}H NMR ($CDCl_3$): δ 1.25 (3H, t, J = 7.5), 2.6 (2H, q, J = 7.5), 7.21–7.24 (2H, m), 7.37–7.40 (3H, m), 7.50–7.53 (2H, d, J = 8.0); IR (KBr): 3099, 2965, 1500, 1428, 1202, 783 cm^{-1}; MS (*m*/*z*): 188 (M^+); mp: 92.25 °C (Lit[88] mp: 96–97 °C). **5**: ^{1}H NMR ($CDCl_3$): δ 2.0 (3H, d, J = 6.9), 5.27 (1H, q, J = 6.9), 7.38–7.48 (5H, m), 7.56 (2H, d, J = 8.1); IR (KBr): 3097, 2982, 1428, 1372, 1174, 783 cm^{-1}; MS (*m*/*z*): 266 $(M-1)^+$; mp: 88 °C. **6**: ^{1}H NMR ($CDCl_3$): δ 1.67 (3H, d, J = 7.3), 3.92 (1H, q, J = 7.3), 7.37–7.47 (5H, m), 7.6 (2H, d, J = 8.2); IR (KBr): 3100, 2984, 2239, 1500, 837, 782 cm^{-1}; MS (*m*/*z*): 213 (M^+); mp: 99.5 °C (Lit[87] mp: 110 °C). **1b**: ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, d, J = 7.1), 3.76 (1H, q, J = 7.1), 7.3–7.42 (5H, m), 7.55 (2H, d, J = 8.1); IR (KBr): 3101, 2936, 1691, 1408, 1223, 776 cm^{-1}; MS (*m*/*z*): 232 (M^+); mp: 186.12 °C (Lit[87] mp: 182 °C).
16. 0.5 g (2.1 mmol) and 0.42 (2.1 mmol) were dissolved in 5 mL of methanol and the solvent evaporated. The resulting diastereomeric salt was dissolved in 10 mL of absolute ethanol and kept for crystallization. The crystals were dissolved in 3.5% HCl (10 mL) and extracted with diethylether (2×10 mL). The ethereal layer was dried over Na_2SO_4 and evaporated to dryness to give *S*-**1b**. The ratio of the two enantiomers of **1b** was determined by (*R*,*R*) Whelk-O 1 with 98/2/0.1 hexane/isopropyl alcohol/acetic acid as mobile phase using UV detector at 264 nm. The retention time of *S* and *R* enantiomers of **1b** was 13.9 min and 14.9 min, respectively.
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