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Full-length article

Design, synthesis, and anti-inflammatory evaluation of a series of novel amino acid-binding 1,5-diarylpyrazole derivatives

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Key words

nonsteroidal anti-inflammatory agents; cyclo oxygenase inhibitors; celecoxib; pyrazole; sulfonamide; prodrugs

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Abstract

Aim: To design and synthesize a series of novel amino acid-binding 1,5-diarylpyrazole derivatives, which are intended to act as prodrugs with better aqueous solubility than celecoxib, and which will exert potent anti-inflammatory activities after being converted to their parent compounds *in vivo*. **Methods:** To introduce an amino acid, celecoxib analogs containing amino or methylamino group were synthesized first through multi-step chemical reactions. All the synthesized compounds were screened in an intact cell-based assay *in vitro* and in carrageenan-induced mouse paw edema *in vivo*. Some active compounds were selected for further evaluation in a carrageenan-induced rat paw edema model. The preliminary pharmacokinetics experiments were conducted using high performance liquid chromatography/mass spectrometry (HPLC/MS). **Results:** Celecoxib, 6 of the 1,5-diarylpyrazole class of celecoxib analogs, and their amino acid derivatives (hydrochloride salts) were synthesized. *In vitro* screening, the hydrochloride salts showed decreased inhibitory effects on cyclooxygenase (COX)-1 and COX-2 compared with their parent compounds, but some exhibited potent anti-inflammatory activity *in vivo*. Compound 4a was selected for further evaluation, and its anti-inflammatory effect was equivalent to that of celecoxib after oral administration in the carrageenan-induced rat paw edema model. At three doses (25 mg/kg, 50 mg/kg, and 100 mg/kg) the percentage inhibition on edema was 20.7%, 52.6%, and 62.6% (for compound 4a) and 27.8%, 38.4%, and 40.1% (for celecoxib), respectively. Preliminary pharmacokinetic evaluations support the hypothesis that compound 4a was actually converted to its parent compound, compound 4. **Conclusion:** The compound bound with amino acid acts like prodrug, which can exert anti-inflammatory effect similar to celecoxib after being converted to its parent compound. This finding will be of great benefit in carrying out structural modifications of prodrug-like selective COX-2 inhibitors.

Introduction

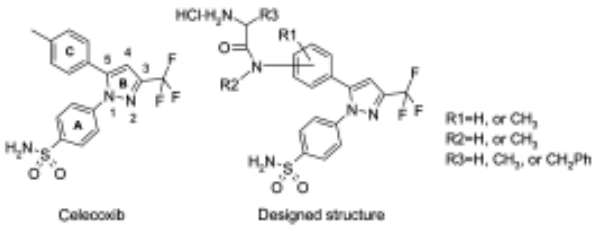
Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain and inflammation. NSAIDs act by inhibiting the catalytic activity of cyclooxygenase (COX), which results in a blockage of the formation of prostaglandins (PGs). COX exists as two distant isoforms (COX-1 and COX-2)[1,2]. Inhibition of COX-2 accounts for the anti-inflammatory effects of NSAIDs, whereas interruption of COX-1 leads to gastrointestinal toxicity[2,3]. Therefore, it is proposed that a selective COX-2 inhibitor would have a superior safety profile[4] to traditional NSAID.

Since the discovery of COX-2, a large number of selective COX-2 inhibitors have been described[5–8], of which vicinal diaryl heterocycles represent the most important group[9–12]. These selective COX-2 inhibitors have pronounced anti-

inflammatory effects with reduced or no gastrointestinal side effects. Celecoxib, in the 1,5-diarylpyrazole class of compound[13], was the first launched selective COX-2 inhibitor, and has excellent selectivity and potent anti-inflammatory activity; however, its aqueous solubility is relatively low, which decreases its oral bioavailability[14]. One approach to address this problem is to convert the compound into a prodrug that is readily soluble in water. Researchers attempted to create a water-soluble form by *N*-acetylation of the sulfonamide group of celecoxib followed by preparation of its sodium salt[15], but the result was unsatisfactory. The acetylation product had a lower level of inhibitory effect on rat paw edema than that produced by celecoxib.

Recently, the cardiovascular safety of coxibs has raised serious concerns; Merck & Co even voluntarily withdrew a rofecoxib product from the market because of an increased risk of cardiovascular events. Although there has been concern about adverse cardiovascular events associated with the use of selective COX-2 inhibitors[16], there is still no evidence indicating that cardiac toxicity is linked with all COX-2 inhibitors[17,18]. Selective COX-2 inhibitors are still under development[5,19,20]. It has been proposed that drugs with higher selectivity for COX-2 tend to induce cardiovascular disease[18,21]. Therefore, we chose the moderately selective COX-2 inhibitor, celecoxib, as a target for modification (the COX-1/COX-2 IC_{50} ratios of etoricoxib, rofecoxib, valdecoxib and celecoxib are 106, 35, 30, and 7.6, respectively).

To improve the oral absorption of celecoxib, a prodrug strategy was used in this study. In our experiments, we were mindful of the following: (1) conserving the main structure of celecoxib, including the trifluoromethyl and benzenesulfonamide groups, which contribute to its inhibitive qualities and selectivity of COX-2[13]; (2) partially containing the methyl group in cycle C, because it can be metabolized to the corresponding hydroxymethyl and carboxylic acid analogs (glucuronide conjugation of the carboxylic acid metabolite is a major pathway of elimination in humans[22]); (3) introducing some polar groups, such as amino or methylamino groups, into cycle C; (4) binding with a natural amino acid followed by preparation of its hydrochloride acid salt. The amide bonds are inclined to be hydrolyzed *in vivo*, and the cleaved natural amino acid part is safe for humans.



Structure of celecoxib and the designed compounds

Based on these considerations, in the present study, we designed and synthesized some 1,5-diarylpyrazole derivatives. These hydrochloride salts were expected to have good aqueous solubility and act like prodrugs, which can exhibit potent anti-inflammatory activity *in vivo* after being converted to their parent compounds.

Materials and methods

Animals Female Kun-ming mice (grade SPF II, certificate No SCXK 2003-0003) weighing 18–22 g and male Sprague-Dawley (SD) rats (grade SPF II, certificate No SCXK 2003-0003) weighing 180–200 g were provided by the Shanghai Experimental Animal Center, Chinese Academy of Sciences. These animals were fasted with free access to water for at least 12 h prior to the experiments. All the animal treatments were strictly in accordance with the Chinese National Institute of Health's Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Chemicals and reagents Substituted acetophenone and ethyl trifluoracetate were purchased from Aldrich (St Louis, MO, USA) and Acros (Geel, Belgium). The tert-butoxycarbonyl (BOC)-protected amino acid was from GL Biochem (Shanghai, China). The other reagents were from the Shanghai Chemical Reagent Co (Shanghai, China). All the chemicals were analytical grade or above.

Chemistry To obtain the expected prodrugs, celecoxib (positive drug), and celecoxib analogs (parent compounds) containing amino or methylamino groups were synthesized first as follows.

4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (celecoxib) Celecoxib (compound 2) was synthesized as described previously[13] with some modifications. In first step, sodium methanol was replaced with sodium ethanol, and the reaction time was reduced from 24 h to 4 h. In second step, dione 1 reacted with (4-sulfamoylphenyl)hydrazine hydrochloride (self-preparation) to produce celecoxib in the form of white crystals (Scheme 1, a-b). Purity as assessed by high performance liquid chromatography (HPLC) was 99.8%. The structure was identified by EI-MS, Mp, and H^1NMR.

Celecoxib analogs containing amino or methylamino groups (parent compounds) Three classes of 6 compounds were synthesized as followed.

1. 4-[5-(3-Amino-4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (compound 4) Celecoxib was treated with diluted HNO_3 2 mol/L and concentrated H_2SO_4 to give the nitration product 3. Then the nitro group was reduced to an amino group by 10% Pd/C

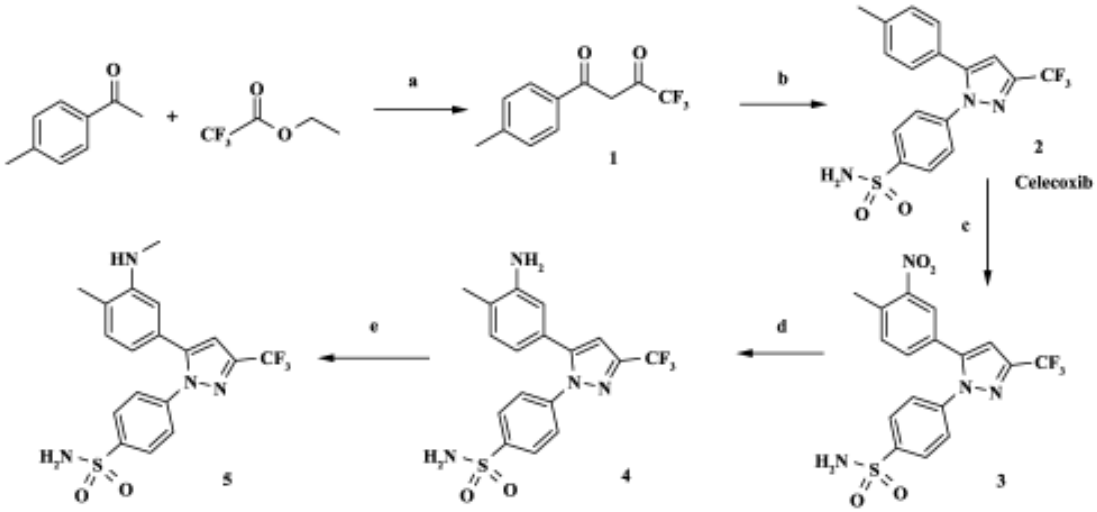
and H_2 to obtain compound 4. To introduce one methyl group on the N atom of the amino group, the N-formyl amine was obtained by the *N*-formylation of compound 4 with formic acid, then it was reduced by lithium aluminum hydride ($LiAlH_4$)[23] to give the desired *N*-monomethyl aromatic amine compound 5 (Scheme 1, c-e).

2. 4-[5-(3-Methyl-4-aminophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (compound 9) 3-Methyl-4-nitro acetophenone (compound 6) as a starting material was prepared as described previously[24] (Scheme 2, a–c). We tried the same method as used in the preparation of celecoxib, but did not obtain the desired compound 7. After different bases and solvents were experienced in step d, we found that the presence of benzene and tert-butanol potassium were suitable for dione (compound 6) synthesis, and that the temperature should be kept at less than 0 °C. Then compounds 9 and 10 were produced (Scheme 2, d-f). by similar method as Scheme 1.

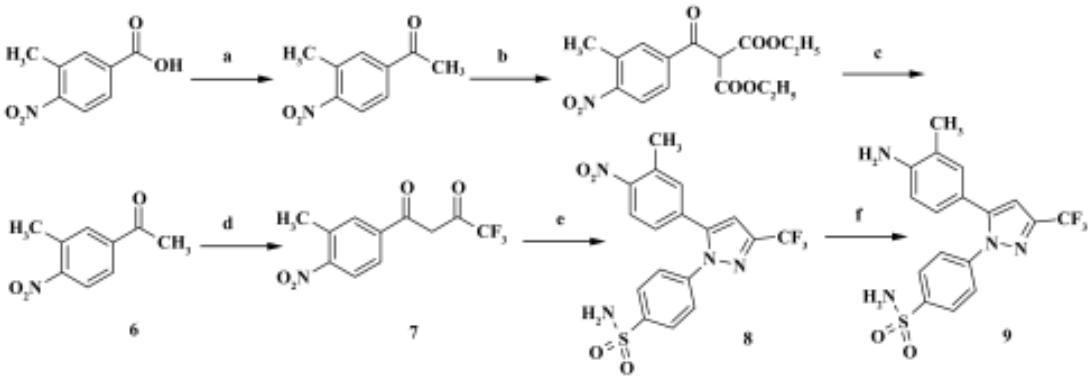
3. 4-[5-(4-Aminophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (compound 11) 4-Nitro acetophenone was used as the starting material. Compounds 11 and 12 were synthesized in the same way as compounds 9 and 10.

All the 6 compounds were identified by EI-MS, Mp, and H^1NMR, and their purities as determined by HPLC were all above 99.3%.

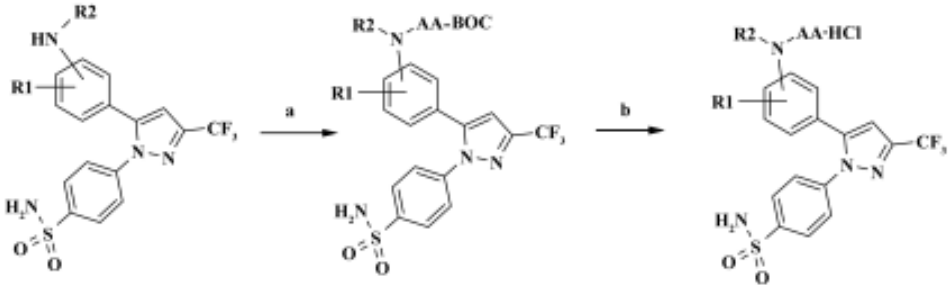
Synthesis of prodrug derivatives The 3 classes of celecoxib analogs containing amino or methylamino groups reacted with several kinds of tert-BOC-protected amino acids in the presence of 1-(3-dimethylaminopropyl)-3-ethyl-



Scheme 1. (a) NaOEt/EtOH, N_2, refluxed for 4 h, yield 89%; (b) (4-sulfamoylphenyl)hydrazine hydrochloride, EtOH, refluxed for 6 h, yield 84%; (c) 2N HNO_3/H_2SO_4, -5–0 °C, 0.5 h, yield 79%; (d) 10% Pd/C, H_2, EtOH, rt, 5 h, yield 98%; (e) i. HCOOH, toluene, refluxed for 3 h, yield 98%; ii. $LiAlH_4$/THF, -5–0 °C, 2 h, yield 57%.



Scheme 2. (a) PCl_5, benzene, refluxed for 2 h; (b) $C_2H_5OMg{\cdot}CH$ $(COOC_2H_5)_2/Et_2O$/EtOH/benzene, refluxed for 0.5 h; (c) $HOAc/H_2SO_4/H_2O$, refluxed for 5 h. (a–c: overall yield 55%–65%); (d) $CF_3COOC_2H_5$, *t*-BuOK, benzene, ice-water bath, 3 h, yield 78%; (e) (4-sulfamoylphenyl) hydrazine hydrochloride, EtOH, refluxed for 5 h, yield 89%; (f) 10%Pd/C, H_2, EtOH, rt, 3 h, yield: 90%.



Scheme 3. (a) EDCI, BOC-AA-OH, THF, rt, overnight, yield 67%–92%; (b) saturated HCl-$CH_3COOC_2H_5$ solution, rt, overnight, yield 82%–93%. R_1= H or CH_3; R_2= H or CH_3; AA = amino acid.

carbodiimide hydrochloride (EDCI). Then the intermediates bound with the BOC group were deprotected with saturated hydrochloride-ethyl acetate solution to obtain the final amino acid hydrochloride salt (Scheme 3). All products were checked by EI-MS, Mp, H^1NMR, IR, and HRMS.

Biological assays

In vitro biochemical assays Cell-based assays were performed as described previously[25]. In brief, the drugs were pre-incubated with infected *Spodoptera frugiperda* (Sf9) cells for 15 min prior to a 10 min challenge with 10 μmol/L arachidonic acid. The concentration of prostaglandin E2 (PGE_2) was determined by interpolation from a standard curve and inhibition was calculated by comparison of PGE_2 production by drug-treated cells with that of Me_2SO-treated cells.

In vivo screening methods. Carrageenan-induced rat paw edema[26] Test compounds were suspended in 0.5% carboxymethylcellulose solution and intragastrically administered to rats at three doses (25 mg/kg, 50 mg/kg and 100 mg/kg) before carrageenan was injected. The control group rats received the same volume of vehicle (0.5% carboxymethylcellulose solution) according to their weight. Paw edema was then induced by subplantar injection of 50 μL 1% (w/v) sterile carrageenan in saline into the right hindpaw. Paw volume was measured before and 3 h after carrageenan injection with a plethysmometer (Shandong Academy of Medical Sciences, China). The extent of paw edema in the treatment group was compared to that of the vehicle control group and percentage inhibition was calculated in comparison to the vehicle group.

In the preliminary screening of mice *in vivo*, the inflammatory index was paw weight instead of volume, which was used for the rats.

Preliminary pharmacokinetic study of prodrugs As described previously[27], HPLC/MS was used to confirm the conversion of prodrug-like derivatives *in vivo* in rats. A single dose (100 mg/kg) of the selected compound (that showed potent inhibitory effects on rat paw edema), was orally administered to rats (n=6). Blood samples (50 μL) were taken before and 0.5 h, 2 h, 6 h, 12 h, 18 h, 24 h, 30 h, 36 h, 48 h, 60 h, and 72 h after intragastric administration, and immediately centrifuged at 10 000×g at 4 °C for 15 min. The plasma samples were stored at -20 °C.

Plasma concentration-time curves were evaluated by InnaPhase Kinetica (InnaPhase Corporation, Philadelphia, PA, USA)

Statistical analysis Data were expressed as mean±SEM (unless noted otherwise), and subjected to one-way analysis of variance (ANOVA) and Dunnett's test. P<0.05 was considered to be statistically significant.

Results

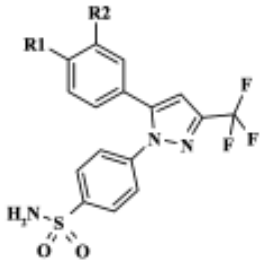
Chemistry Celecoxib, 6 of the 1,5-diarylpyrazole class of celecoxib analogs, and their amino acid derivatives (hydrochloride salts) were synthesized. Three kinds of BOC-protected natural amino acids were selected for introduction. They were glycine, *L*-alanine, and *L*-phenylalanine. The main analytical data, from ^{1}H NMR and HRMS, are summarized in Table 1.

The aqueous solubility of the hydrochloride salts was greatly improved compared with that of their parent compounds and celecoxib. Their aqueous solubilities were, respectively, 10–33 g/L, 0.1–1 g/L, and <0.05 g/L at 18 °C.

***In vitro* biochemical screening** All the compounds were evaluated using the intact cell assay. Parent compounds 4, 5, 9, and 10 exhibited potent and selective inhibition of COX-2 activity *in vitro* (Table 2), whereas the inhibitory activities of the hydrochloride salts were greatly decreased compared with their parent compounds.

***In vivo* biological evaluation** Although none of the hydrochloride salts had any anti-inflammatory activity *in vitro*, they could be expected to exert anti-inflammatory effects once they were converted into their parent compounds.

Table 1. Structure and analytical data (^{1}H NMR and HRMS) of synthesized compounds.



Compound	Substituted group R1	Substituted group R2	^{1}H NMR	HRMS (*m/z*) Found	HRMS (*m/z*) Calculated
Celecoxib	CH_3	H	($CDCl_3$): d 7.90 (dd, *J*=6.8,2.0 Hz, 2H), 7.6 (d, *J*=6.5 Hz, 2H), 7.46 (dd, *J*=6.8,2.0 Hz, 2H), 7.19 (d, *J*=6.5 Hz, 2H), 6.78 (s, 1H), 4.90 (s, 2H), 2.0 (s, 3H).	–	–
4	CH_3	NH_2	($CDCl_3$): δ 7.89 (ddd, *J*=8.8,2.3,2.3 Hz, 2H), 7.5 (ddd, *J*=8.8, 2.3,2.3 Hz, 2H), 7.02 (d, *J*=7.7 Hz, 1H), 6.70 (s, 1H), 6.54 (d, *J*=1.5 Hz, 1H), 6.49 (dd, *J*=7.7,1.5 Hz, 2H), 4.99 (s, 2H), 2.19 (s, 3H).	–	–
5	CH_3	NHMe	($CDCl_3$): δ 7.89 (ddd, *J*=8.93,2.2,2.2 Hz, 2H), 7.51 (ddd, *J*= 8.93,2.2,2.2 Hz, 2H), 7.0 (dd, *J*=7.56,0.42 Hz, 1H), 6.75 (s, 1H), 6.45 (dd, *J*=7.56, 1.70 Hz, 1H), 6.39 (d, *J*=1.70 Hz, 1H), 4.9 (s, -SO_2NH_2, 2H), 3.7 (s, -N<u>H</u>CH_3, 1H), 2.75 (s, -NHC<u>H$_3$</u>, 3H), 2.15 (s, C<u>H$_3$</u>-Ar, 3H).	–	–
11	NH_2	H	($CDCl_3$): δ 7.89 (dd, *J*=6.83,1.95Hz, 2H), 7.48 (dd, *J*=6.83,1.95 Hz, 2H), 6.94 (m, 2H), 6.64 (s, 1H), 6.61 (m, 2H), 4.84 (s, -SO_2NH_2, 2H), 3.84 (s, 2H).	–	–
12	NHMe	H	($CDCl_3$): δ 7.92 (d, *J*=8.8 Hz, 2H), 7.52 (d, *J*=8.8 Hz, 2H), 7.02 (m, 2H), 6.64 (s, 1H), 6.56 (m, 2H), 4.90 (s, -SO_2NH_2, 2H), 2.86 (s, 3H).		
9	NH_2	CH_3	(Me_2SO-d_6): δ 7.85 (ddd, *J*=8.80,2.20,2.20 Hz, 2H), 7.52 (ddd, *J*= 8.80, 2.20,2.20 Hz, 2H), 6.97 (s, 1H), 6.95 (d, *J*=1.4 Hz, 1H), 6.65 (dd, *J*=8.24, 2.2 Hz, 1H), 6.50 (d, *J*=8.24 Hz, 1H), 1.99 (s, 3H).	396.0861	396.0868
10	NHMe	CH_3	-	410.1026	410.1024
4a	CH_3	NH-Gly·HCl	(Me_2SO-d_6): δ 9.92 (s, 1H), 8.14 (s, 3H), 7.84 (m, 2H), 7.50–7.58 (m,5H), 7.26 (d, *J*=8.06 Hz, 1H), 7.14 (s, 1H), 7.01 (d, *J*=8.07 Hz, 1H), 3.80 (d, *J*=5.50 Hz, 2H), 2.22 (s, 3H).	453.1076	453.1083
4b	CH_3	NH-Ala·HCl	(Me_2SO-d_6): δ 10.08 (s, 1H), 8.23 (s, 3H), 7.84–7.87 (m, 2H), 7.52–7.55 (m, 2H), 7.51–7.52 (s, 2H), 7.42 (d, *J*=1.68 Hz, 1H), 7.25–7.28 (d, *J*=7.94 Hz, 1H), 7.15 (s, 1H), 7.0–7.03 (dd, *J*=7.94, 1.68 Hz, 1H), 4.10–4.13 (m, 1H), 2.01 (s, 3H), 1.42 –1.44 (d, *J*=6.87 Hz, 3H).	467.1241	467.1239
4c	CH_3	NH-Phe·HCl	(Me_2SO-d_6): δ 9.92 (s, 1H), 8.40 (s, 2H), 7.84–7.87 (d, *J*=8.78 Hz, 2H), 7.52–7.55 (d, *J*=8.80 Hz, 2H), 7.51 (s, -SO_2NH_2, 2H), 7.25–7.32 (m, 6H),7.21–7.23 (d, *J*=8.06 Hz, 1H), 7.10 (s, 1H), 6.99–7.01 (m, 1H), 4.30 (m, 1H), 3.06–3.08 (d, *J*=7.15 Hz, 1H), 2.01(s, 3H).	543.1535	543.1552
5a	CH_3	NMe-Gly·HCl	(Me_2SO-d_6): δ 7.88–7.91 (m, 2H), 7.52–7.56 (d, *J*=8.80 Hz, 2H), 7.42–7.44 (d, *J*=8.07 Hz, 1H), 7.29–7.32 (m, 2H), 7.18 (d, *J*=1.47 Hz, 1H), 3.0 (s, 3H), 2.2 (s, 3H).	467.1254	467.1239
11a	NH-Gly·HCl	H	(Me_2SO-d_6): δ 10.82 (s, 1H), 8.14–8.24(m, 2H), 7.82–7.84(d, *J*=8.79 Hz, 2H), 7.60(d, *J*=8.79 Hz, 2H), 7.50–7.54(m, 4H),	439.0924	439.0926

Compound	Substituted group R1	R2	^{1}H NMR	HRMS (*m*/*z*) Found	Calculated
11b	NH-Ala·HCl	H	7.26 (d, *J*=8.79 Hz, 2H), 7.18 (s, 1H), 3.74(s, 2H). (Me_2SO-d_6): δ 7.85–7.87 (d, *J*=8.61 Hz, 2H), 7.65–7.67(d, *J*= 8.80 Hz, 2H), 7.51–7.53 (m, 4H), 7.26–7.28 (d, *J*=8.80 Hz, 2H), 7.19 (s, 1H), 3.90–3.96 (d, *J*=6.97 Hz, 1H), 1.40 (d, *J*=6.78 Hz, 3H).	453.1086	453.1083
11c	NH-Phe·HCl	H	(Me_2SO-d_6): δ 10.99 (s, 1H), 8.37 (s, 1H), 7.85–7.88 (d, *J*=8.80 Hz, 2H), 7.56-7.59 (d, *J*=8.80 Hz, 2H), 7.51–7.54 (d, 4H), 7.26–7.30 (m, 7H), 7.20 (s, 1H), 4.22 (d, *J*=5.14 Hz, 1H), 3.14–3.20(m, 2H).	529.1380	529.1396
12a	NMe-Gly·HCl	H	(Me_2SO-d_6): δ 8.18 (s, 2H), 7.88–7.90 (d, *J*=8.8 Hz, 2H), 7.58–7.44 (m, 8H), 7.28 (s, 1H), 3.22(s, 3H).	453.1071	453.1083
9a	NH-Gly·HCl	CH_3	(Me_2SO-d_6): δ 9.96 (s, -NH_2), 8.23 (s, -NH-), 7.87–7.89 (d, *J*=8.40 Hz, 2H), 7.55–7.57(d, *J*=8.59 Hz, 2H), 7.53–7.55 (m, 1H), 7.33 (m, 1H), 7.20 (s, 1H), 7.02–7.05 (dd, *J*=8.27,1.57 Hz, 1H), 3.82 (s, 2H), 2.0 (s, 3H).	453.1088	453.1083
9b	NH-Ala·HCl	CH_3	(Me_2SO-d_6): δ 7.87–7.90 (ddd, *J*=8.61,2.20,2.20 Hz, 2H), 7.55–7.57 (m, 2H), 7.47–7.49 (d, *J*=8.41 Hz, 1H), 7.33 (d, *J*=1.76 Hz, 1H), 7.20 (s, 1H), 7.02–7.05 (dd, *J*=8.21,2.20 Hz, 1H), 4.10–4.13 (m, 1H), 2.0 (s, 3H), 1.43 (d, *J*=7.05 Hz, 3H).	467.1216	467.1239
9c	NH-Phe·HCl	CH_3	(Me_2SO-d6): δ 7.87–7.89 (m, 2H), 7.54–7.57 (m, 2H), 7.27–7.37 (m, 7H), 7.21 (s, 1H), 6.99–7.02 (m, 1H), 4.35 (m, 1H), 3.10–3.12 (d, *J*=7.22 Hz, 2H), 2.0 (s, 3H).	543.1537	543.1552

"-"= not determined.

Table 2. Inhibitory effect of test compounds on COX-1 and COX-2 *in vitro*.

Compound	IC_{50}/ μmol·L^{-1} COX-2	COX-1
Celecoxib	0.053	2.4
Compound 4	0.33	14.34
Compound 5	0.12	1.51
Compound 9	3.12	6.32
Compound 10	0.46	1.76

Average of at least three determinations, and the deviation from the mean is <10%.

Table 3. *In vivo* data for compounds celecoxib (positive drug), compound 4 (parent compound), and compound 4a (prodrug compoud) on carrageenan-induced rat paw edema. *n*=8. Mean±SEM. $^bP<0.05$, $^cP<0.01$ *vs* control vehicle groups.

Dose /mg·kg^{-1}	Inhibition/% Celecoxib	Compound 4	Compound 4a
25	27.76±4.35^b	26.63±6.47^b	20.68±5.44^b
50	38.4±3.87^b	57.41±2.83^b	52.55±3.26^b
100	40.12±4.45^b	67.29±3.69^b	62.55±3.48^c

Based on our preliminary biological evaluation in mice (data not shown), compound 4a was selected for further evaluation in carrageenan-induced rat paw edema because of its potent inhibitory effect on edema. Three doses (25 mg/kg, 50 mg/kg and 100 mg/kg) of each of celecoxib, compound 4, and compound 4a, respectively, were orally administered to rats. Compound 4a had a high level of efficiency, which was identical to that of the possible parent compound 4 and similar to that of celecoxib (Table 3). Furthermore, compound 4a dose-dependently inhibited the inflammatory response to carrageenan-induced edema.

Preliminary pharmacokinetic study A preliminary pharmacokinetic study of compound 4a in rats (*n*=6) was undertaken to prove that it was converted *in vivo* to compound 4. Figure 1 shows the representative chromatograms of blank plasma, plasma containing compound 4, compound 4a, and internal standard (IS), and plasma sample added with IS 6 h after intragastric administration. The retention times of compound 4a, compound 4, and IS were 5.71 min, 7.91 min, and 6.56 min in blank plasma, respectively. In a plasma sample 6

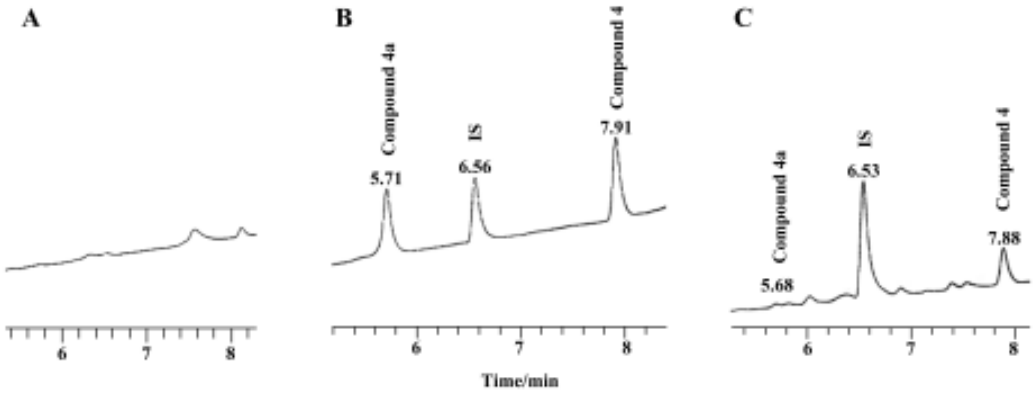


Figure 1. Representative chromatograms of (A) blank plasma; (B) plasma containing compound 4, compound 4a, and internal standard (IS); (C) plasma sample added with IS 6 h after intragastric administration.

h after intragastric administration, the retention times of compound 4a, compound 4 created from the conversion of compound 4a, and IS were 5.68 min, 7.88 min, and 6.53 min, respectively. The corresponding chromatography peak was identified by MS. We thus proved that compound 4a was actually converted to compound 4 after oral administration.

The pharmacokinetic parameters of compound 4 released from compound 4a were calculated by non-compartmental model analysis based on the drug concentration-time curve of 0–72 h sample dotting. Peak plasma concentration (C_{max}), the corresponding time (T_{max}), the area under the plasma concentration-time curve ($AUC_{0-\infty}$), and $T_{1/2}$ were 7.7±1.1 mg/L, 13.2±2.7 h, 165.3±21.8 mg·L^{-1}·h, and 6.4±1.3 h, respectively.

Discussion

In the present study, a series of amino acid-binding 1,5-diarylpyrazole derivatives were designed and synthesized as prodrugs. As expected, the aqueous solubility of these compounds was greatly improved compared with celecoxib. For example, the solubility of compound 4a in water at 18°C was 15–18 g/L, whereas that of celecoxib was <0.05 g/L. Good aqueous solubility should enhance the dissolution rate of a drug and thus improve its oral bioavailability[14].

In *in vitro* biological evaluations, it was found that the parent compounds 4, 5, 9, and 10 exhibited potent selectivity and inhibition of COX-2, although they all did not exceed celecoxib (Table 2). From the results, it was also found that the compounds containing the methylamino group had more potent inhibitory effects than those containing the amino group. In addition, all the hydrochloride salts showed extremely weak inhibition on COX activity.

Based on a previous hypothesis, the hydrochloride salt should bring about beneficial anti-inflammatory effects after delivering its parent compound *in vivo*. Therefore, anti-inflammatory evaluation *in vivo* was performed in carrageenan-induced mouse and rat paw edema models. Compound 4a brought about the expected potent inhibition of edema after intragastric administration. Furthermore, at doses of 50 mg/kg and 100 mg/kg, the anti-inflammatory effect of compound 4a was a little stronger than that of celecoxib, and identical to that of compound 4 (Table 3). Preliminary pharmacokinetic studies in rats also proved that compound 4a actually acted as a prodrug, which has an anti-inflammatory effect once it is converted into its active form, compound 4.

In the preliminary pharmacokinetic studies, the T_{max} of compound 4 (as derived from compound 4a) was found at 12 h after oral administration; however, the T_{max} of celecoxib was at 3 h after administration[28]. In our experiments, we only observed the inhibitory effects on edema at 3 h after oral administration. Therefore we predicted that when the concentration of compound 4 (as derived from compound 4a) reached a maximum, it should have stronger anti-inflammatory activity.

Although compound 4a did not exhibit more potency *in vivo* than its parent, compound 4, its potency was comparable with that of celecoxib (Table 3). We suppose that compound 4a was not completely converted to its parent compound, compound 4, or it produced other metabolisms *in vivo*. Similar prodrug conversion pharmacokinetics have been investigated by Mamidi *et al*[28]. Prodrugs derived from the same parent compound will have different activity *in vivo* when they are connected to different groups. Connecting more amino acids to the parent compound might offer a an exciting prospect for developing prodrug-like NSAIDs.

In summary, by introducing an amino or methylamino group, 6 celecoxib analogs were synthesized as parent compounds. The 6 compounds were combined with three

kinds of natural amino acids to obtain a series of novel hydrochloride salts. By biological evaluation *in vitro* and *in vivo*, and by further preliminary pharmacokinetic studies, compound 4a was discovered, which was confirmed to act as a prodrug, and exhibited marked anti-inflammatory activity *in vivo*. This finding will be of great benefit to the structural modifications of prodrug-like selective COX-2 inhibitors.

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