



Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of amide derivatives of diflunisal as potential anti-inflammatory agents

Guang-Xiang Zhong^{*}, Jin-Qing Hu, Kun Zhao, Lu-Lu Chen, Wei-Xiao Hu, Ming-You Qiu

College of Pharmaceutical Science, Zhejiang University of Technology, 18, Chaowang Road, Hangzhou, Zhejiang 310032, China

ARTICLE INFO

Article history:

Received 21 July 2008

Revised 2 November 2008

Accepted 11 November 2008

Available online 14 November 2008

Keywords:

Diflunisal

Amide derivative

Anti-inflammatory and analgesic activity

ABSTRACT

To improve the medicinal activity, the structure of diflunisal has been modified. Twenty-one amide derivatives of diflunisal were synthesized starting from diflunisal in three steps with total yields from 72% to 89%. All compounds were identified by ¹H NMR, MS, and elemental analysis. The anti-inflammatory and analgesic activities for 19 compounds were evaluated. It was found that **5m** possesses an excellent anti-inflammatory activity and a good analgesic activity, maybe a potential anti-inflammatory agent.

© 2008 Elsevier Ltd. All rights reserved.

Fluorine-containing non-steroid anti-inflammatory drugs have been attractive for their special properties.^{1–3} Diflunisal (**1**, CAS 22494-42-4), 2',4'-difluoro-4-hydroxybiphenyl-3-carboxylic acid, has been approved worldwide as therapeutics for the treatment of inflammation and pain.^{4,5} Recently, the modifications of the chemical structure of **1** were studied. For example, 3-(1,3-dihydro-2H-isoindol-2-ylcarbonyl)-2',4'-difluorobiphenyl-4-ol was synthesized and reported to have the H3P-90 inhibition, and H3P-90 in abnormal cells, such as in cancer cells would damage the regulation of signal transduction network.⁶ Yu reported that esterification or amidation of **1** could increase their solubility and absorption in vivo, and some of them have even better analgesic activity than that of diflunisal.⁷ Some changes in the carboxyl group of **1** also showed good antimycobacterial, antiviral and antimicrobial activities.⁸ Roberts found that *O*-aryl esters of **1**, especially lipophilic esters, possess large permeability surface area and tissue distribution value.⁹

Our group have studied the synthesis and the SAR of fluorine-containing non-steroid anti-inflammatory drugs, and patented that some amides of fluorine-containing benzoic acid possessed good anti-inflammatory activity.^{10,11}

In continuation of our research, the modification of diflunisal was studied by esterification of the hydroxyl group and amidation of the carboxylic group. We designed and synthesized 17 amide derivatives of *O*-acetyldiflunisal or *O*-benzoyldiflunisal, **5a–5q**, and evaluated their anti-inflammatory and analgesic activity. Accidentally, four amide derivatives of **1**, **5r–5u**, were obtained in the

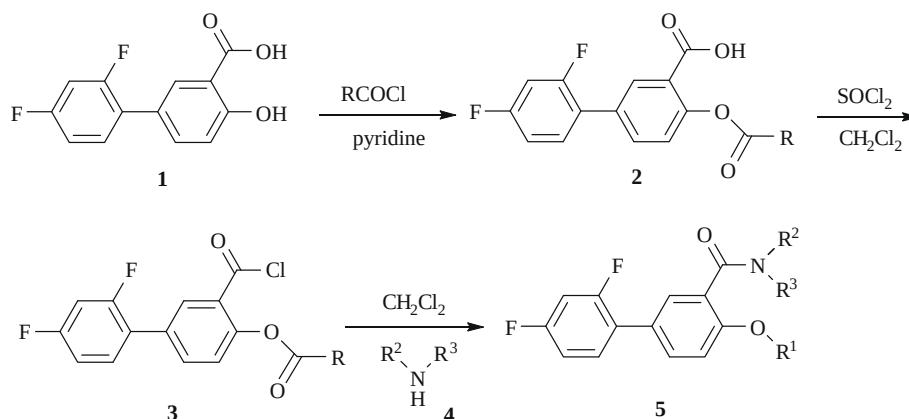
same way when **3a** (R = CH₃) was reacted with a secondary amine in amidation, such as dimethylamine, diethylamine, *N*-methylpiperazine or *N*-ethylpiperazine, because the four secondary amines are stronger alkaline and promoted the hydrolyzation of acetates. The 21 amides were synthesized from 72% to 89% in total yield. The synthetic route is shown in Scheme 1. Starting from **1**, esterification of phenolic hydroxy group and amidation of carboxylic acid gave desired product **5**. The preparations are summarized in Table 1. The structures of all compounds were identified by IR, ¹H NMR, MS, and elemental analysis (Table 2).^{12,13} The crystal structure of **5a** was determined by X-ray.¹⁴

The anti-inflammatory and analgesic activities for these compounds (**2**, **5a–5q**) were evaluated by xylene induced mice ear edema and acetic acid induced mice writhing models for male and female Kunming mice (weight 20–26 g). The substances were administrated via the oral route at the dose of 40 mg kg^{−1}. The diflunisal, a registered anti-inflammatory drug, ED₅₀ 59.6 mg kg^{−1} on carrageenin-induced paw edema in rats,^{15,16} was used as a positive control. The results of the evaluation of anti-inflammatory and analgesic activity are summarized on Table 3. The ED₅₀ on active compounds was determined and the results were listed in Table 4. The ED₅₀ for **5m** and **5p** on xylene induced mice ear edema model is 24.24 and 25.69 mg kg^{−1} and for **5m** and **5q** on acetic acid induced mice writhing model is 43.79 and 11.58 mg kg^{−1}.

From Table 3, it could be found that the inhibition on xylene induced mice ear edema model of **5m**, **5p**, **5a**, and **5i** is 68.86%, 65.20%, 48.83%, and 41.37% relatively, superior to diflunisal 39.85% at the dose of 40 mg kg^{−1}. Comparing **2b**, **5m**, **5n**, and **5o** with **2a**, **5a**, **5e**, and **5f** relatively, the biological activity of **5m** is superior to **5a** in both anti-inflammatory and analgesic activity

^{*} Corresponding author. Tel.: +86 571 88320722; fax: +86 571 88320557.

E-mail address: drzhonggx@sohu.com (G.-X. Zhong).



Scheme 1. Route of synthesis.

Table 1
Synthesis of compounds (**5**)

Compound	R ¹	R ²	R ³	Yield (%)	Mp (°C)
5a	COCH ₃	H	CH ₃	81	157–160
5b	COCH ₃	H	CH ₂ CH ₃	77	110–112
5c	COCH ₃	H	Cyclohexyl	73	159–162
5d	COCH ₃	H	CH ₂ C ₆ H ₅	81	134–136
5e	COCH ₃	H	C ₆ H ₅	87	152–155
5f	COCH ₃	H	<i>o</i> -C ₆ H ₄ CH ₃	85	166–169
5g	COCH ₃	H	<i>m</i> -C ₆ H ₄ CH ₃	81	123–125
5h	COCH ₃	H	<i>p</i> -C ₆ H ₄ CH ₃	89	164–167
5i	COCH ₃	H	<i>o</i> -C ₆ H ₄ Cl	84	159–163
5j	COCH ₃	H	<i>p</i> -C ₆ H ₄ Cl	84	183–186
5k	COCH ₃	H	2,5-(CH ₃) ₂ C ₆ H ₃	81	109–112
5l	COCH ₃	H	Morpholine-4-yl	76	124–126
5m	COC ₆ H ₅	H	CH ₃	75	147–150
5n	COC ₆ H ₅	H	C ₆ H ₅	84	139–140
5o	COC ₆ H ₅	H	<i>o</i> -C ₆ H ₄ CH ₃	86	165–168
5p	COC ₆ H ₅	CH ₂ CH ₃	CH ₂ CH ₃	72	125–128
5q	COC ₆ H ₅	H	4-Methylpiperazin-1-yl	83	154–157
5r	H	CH ₃	CH ₃	76	123–125
5s	H	CH ₂ CH ₃	CH ₂ CH ₃	73	139–141
5t	H	H	4-Methylpiperazin-1-yl	74	95–97
5u	H	H	4-Ethylpiperazin-1-yl	77	142–143

and others are nearly equal to each partner. Therefore, the compound containing *O*-benzoyl group may have a good biological activity.

Additionally, it indicates that the substituted groups on amide could affect the anti-inflammatory activity. It was found that the alkyl group benefits as increase as following: CH₃ > CH₂C₆H₅ > CH₂CH₃, and the aryl group are more complex. It was discovered that the compounds with such electron-donating group (CH₃ group) seems to benefit as increase as following: *para* > *ortho* > *meta*, and the compounds with electro-withdrawing group (Cl group) is *ortho* > *para*. But it needs more data to find the relationship.

From Table 3, it could also be found that the inhibition on acetic acid induced mice writhing model of **5q** and **5m** is 88.33% and 47.50% at the dose of 40 mg kg⁻¹. Additionally, it indicates that the substituted groups on amide could also affect the analgesic activity and the compound with hetero-atom on amide group is better than others in analgesic activity. The effect of substituted group on the analgesic activity seems more complex than on the anti-inflammatory activity.

On the whole, it seems a trend that the substituted groups on the either amide or ester could affect on the inflammatory activity

Table 2
Elemental analysis (calcd data in parentheses) and ¹H NMR data (in CDCl₃) of new compounds

Compound	Elemental analysis (%)			¹ H NMR (δ, ppm)
	C	H	N	
5a	62.86 (62.95)	4.36 (4.29)	4.49 (4.59)	2.36 (s, 3H, COCH ₃), 2.99 (d, 3H, <i>J</i> = 4.8 Hz, N-CH ₃), 6.25 (s, 1H, -NH), 6.94 (m, 2H, 3',5'-CH), 7.18 (d, 1H, <i>J</i> = 8.4 Hz, 5-CH), 7.40 (q, 1H, <i>J</i> = 7.6 Hz, 6'-CH), 7.59 (d, 1H, <i>J</i> = 8.4 Hz, 6-CH), 7.84 (s, 1H, 2-CH)
5b	63.83 (63.95)	4.82 (4.74)	4.31 (4.39)	1.24 (t, 3H, <i>J</i> = 7.2 Hz, -CH ₃), 2.34 (s, 3H, -COCH ₃), 3.47 (m, 2H, -CH ₂), 6.25 (s, 1H, -NH), 6.94 (m, 2H, 3',5'-CH), 7.17 (d, 1H, <i>J</i> = 8.4 Hz, 5-CH), 7.40 (q, 1H, <i>J</i> = 7.6 Hz, 6'-CH), 7.59 (d, 1H, <i>J</i> = 8.4 Hz, 6-CH), 7.84 (s, 1H, 2-CH)
5c	67.43 (67.55)	5.73 (5.67)	3.67 (3.75)	1.24 (m, 3H, 3'',4'',5''-CH), 1.44 (q, 2H, <i>J</i> = 5.0 Hz, 3'',5''-CH), 1.66 (m, 1H, 4''-CH), 1.74 (m, 2H, 2'',6''-CH), 2.02 (m, 2H, 2'',6''-CH), 2.36 (s, 3H, -CH ₃), 3.96 (m, 1H, 1''-CH), 6.13 (d, 1H, <i>J</i> = 7.5 Hz, -NH), 6.92 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.96 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.16 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.41 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.58 (d, 1H, <i>J</i> = 8.5 Hz, 6-CH), 7.84 (s, 1H, 2-CH)
5d	69.22 (69.29)	4.55 (4.49)	3.56 (3.67)	2.08 (s, 3H, -CH ₃), 4.61 (d, 2H, <i>J</i> = 5.5 Hz, -CH ₂), 6.63 (s, 1H, N-H), 6.92 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.97 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.16 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.35 (q, 1H, <i>J</i> = 8.5 Hz, 6'-CH), 7.38 (m, 5H, -C ₆ H ₅), 7.59 (s, 1H, 2-CH), 7.94 (d, 1H, <i>J</i> = 8.4 Hz, 6-CH)
5e	68.60 (68.66)	4.19 (4.12)	3.75 (3.81)	2.37 (s, 3H, -CH ₃), 6.94 (m, 2H, 3',5'-CH), 7.17 (d, 1H, <i>J</i> = 8.0 Hz, 5-CH), 7.25 (q, 1H, <i>J</i> = 8.0 Hz, 4''-CH), 7.40 (m, 3H, 6',3'',5''-CH), 7.64 (m, 3H, 6, 2'',6''-CH), 7.98 (s, 1H, 2-CH), 8.07 (s, 1H, -NH)
5f	69.23 (69.29)	4.54 (4.49)	3.60 (3.67)	2.34 (s, 3H, -CH ₃), 2.37 (s, 3H, -CH ₃), 6.95 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 7.00 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.15 (t, 1H, <i>J</i> = 7.5 Hz, 4''-CH), 7.25 (d, 1H, <i>J</i> = 7.5 Hz, 5-CH), 7.27 (d, 1H, <i>J</i> = 8.5 Hz, 6''-CH), 7.28 (t, 1H, <i>J</i> = 8.5 Hz, 5''-CH), 7.46 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.67 (d, 1H, <i>J</i> = 8.5 Hz, 3''-CH), 7.83 (s, 1H, 2-CH), 7.95 (d, 1H, <i>J</i> = 7.5 Hz, 6-CH), 8.00 (s, 1H, -NH)
5g	69.23 (69.29)	4.55 (4.49)	3.58 (3.67)	2.36 (s, 3H, -CH ₃), 2.37 (s, 3H, -CH ₃), 6.95 (m, 2H, 3',5'-CH), 6.99 (d, 1H, <i>J</i> = 7.6 Hz, 4''-CH), 7.24 (d, 1H, <i>J</i> = 8.0 Hz, 5-CH), 7.27 (t, 1H, <i>J</i> = 7.6 Hz, 4''-CH), 7.36 (d, 1H, <i>J</i> = 7.6 Hz, 6''-CH), 7.43 (q, 1H, <i>J</i> = 7.6 Hz, 6'-CH), 7.51 (s, 1H, 2''-CH), 7.65 (d, 1H, <i>J</i> = 8.4 Hz, 6-CH), 7.97 (s, 1H, 2-CH), 8.01 (s, 1H, -NH)
5h	69.21 (69.29)	4.54 (4.49)	3.61 (3.67)	2.36 (s, 3H, -CH ₃), 2.38 (s, 3H, -CH ₃), 6.95 (m, 2H, 3',5'-CH), 7.19 (d, 2H, <i>J</i> = 7.6 Hz, 3'',5''-CH), 7.25 (d, 1H, <i>J</i> = 8.0 Hz, 5-CH), 7.43 (q, 1H, <i>J</i> = 7.6 Hz, 6'-CH), 7.51 (d, 2H, <i>J</i> = 8.0 Hz, 2'',6''-CH), 7.65 (d, 1H, <i>J</i> = 8.4 Hz, 6-CH), 7.99 (s, 1H, 2-CH), 8.01 (s, 1H, -NH)

(continued on next page)

Table 2 (continued)

Compound	Elemental analysis (%)			¹ H NMR (δ , ppm)
	C	H	N	
5i	62.70 (62.78)	3.56 (3.51)	3.43 (3.49)	2.41 (s, 3H, -CH ₃), 6.96 (m, 2H, 3',5'-CH), 7.10 (t, 1H, <i>J</i> = 7.6 Hz, 4''-CH), 7.28 (d, 1H, <i>J</i> = 8.0 Hz, 5-CH), 7.34 (t, 1H, <i>J</i> = 8.0 Hz, 5''-CH), 7.44 (m, 2H, <i>J</i> = 8.0 Hz, 6',3''-CH), 7.68 (d, 1H, <i>J</i> = 8.4 Hz, 6-CH), 8.10 (s, 1H, 2-CH), 8.58 (d, 1H, <i>J</i> = 8.4 Hz, 6''-CH), 8.78 (s, 1H, -NH)
5j	62.71 (62.78)	3.57 (3.51)	3.42 (3.49)	2.36 (s, 3H, -CH ₃), 6.95 (m, 2H, 3',5'-CH), 7.25 (d, 1H, <i>J</i> = 8.0 Hz, 5-CH), 7.34 (d, 2H, <i>J</i> = 8.4 Hz, 3'',5''-CH), 7.43 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.57 (d, 2H, <i>J</i> = 8.4 Hz, 2'',6''-CH), 7.66 (d, 1H, <i>J</i> = 8.0 Hz, 6-CH), 7.97 (s, 1H, 2-CH), 8.06 (s, 1H, -NH)
5k	69.78 (69.87)	4.92 (4.84)	3.43 (3.54)	2.29 (s, 3H, CO-CH ₃), 2.37 (s, 6H, Ph-CH ₃), 6.94 (d, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.96 (d, 1H, <i>J</i> = 8.0 Hz, 4''-CH), 7.00 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.13 (d, 1H, <i>J</i> = 8.0 Hz, 3''-CH), 7.26 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.46 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.67 (d, 1H, <i>J</i> = 8.0 Hz, 6-CH), 7.79 (s, 2H, 2,6''-CH), 8.00 (s, 1H, -NH)
5l	63.04 (63.15)	4.83 (4.74)	3.79 (3.88)	2.32 (s, 3H, -CH ₃), 3.40 (t, 2H, <i>J</i> = 5.0 Hz, 2'',6''-CH), 3.63 (t, 2H, <i>J</i> = 5.0 Hz, 2'',6''-CH), 3.75 (m, 4H, 3'',5''-CH ₂), 6.92 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.97 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.26 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.36 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.43 (s, 1H, 2-CH), 7.55 (d, 1H, <i>J</i> = 8.5 Hz, 6-CH)
5m	68.49 (68.66)	4.14 (4.12)	3.70 (3.81)	2.89 (d, 3H, <i>J</i> = 4.8 Hz, CH ₃), 6.36 (s, 1H, NH), 6.94 (t, 1H, <i>J</i> = 8.4 Hz, 3'-H), 7.00 (t, 1H, <i>J</i> = 8.2 Hz, 5'-H), 7.31 (d, 1H, <i>J</i> = 8.4 Hz, 5-H), 7.45 (q, 1H, <i>J</i> = 7.6 Hz, 7.56 (t, 2H, <i>J</i> = 7.6 Hz, 3'',5''-H), 7.65 (d, 1H, <i>J</i> = 8.4 Hz, 6-H), 7.69 (t, 1H, <i>J</i> = 7.6 Hz, 4''-H), 7.94 (s, 1H, 2-H), 8.27 (d, 2H, <i>J</i> = 6.8 Hz, 2'',6''-H)
5n	72.59 (72.72)	4.01 (3.99)	3.19 (3.26)	6.96 (t, 1H, <i>J</i> = 8.4 Hz, 3'-H), 7.00 (t, 1H, <i>J</i> = 8.4 Hz, 5'-H), 7.08 (t, 1H, <i>J</i> = 7.6 Hz, 4''-H), 7.26 (t, 2H, <i>J</i> = 8.0 Hz, 3'',5''-H), 7.37 (d, 1H, <i>J</i> = 8.0 Hz, 5-H), 7.45 (d, 2H, <i>J</i> = 7.6 Hz, 2'',6''-H), 7.47 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.55 (t, 2H, <i>J</i> = 7.6 Hz, 3'',5''-H), 7.69 (t, 1H, <i>J</i> = 7.6 Hz, 4''-H), 7.72 (d, 1H, <i>J</i> = 8.0 Hz, 6-H), 8.10 (s, 1H, 2-H), 8.23 (s, 1H, NH), 8.24 (d, 2H, <i>J</i> = 7.2 Hz, 2'',6''-H)
5o	73.27 (73.13)	4.40 (4.32)	3.13 (3.16)	2.18 (s, 3H, CH ₃), 6.96 (t, <i>J</i> = 8.4 Hz, 1H, 3'-H), 7.00 (t, 1H, <i>J</i> = 8.0 Hz, 5'-H), 7.07 (t, 1H, <i>J</i> = 7.2 Hz, 4''-H), 7.14 (d, 1H, <i>J</i> = 7.6 Hz, 3'''-H), 7.20 (t, 1H, <i>J</i> = 6.8 Hz, 5'''-H), 7.36 (d, 1H, <i>J</i> = 8.4 Hz, 5-H), 7.48 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.53 (t, 2H, <i>J</i> = 7.6 Hz, 3'',5''-H), 7.68 (t, 1H, <i>J</i> = 7.6 Hz, 4''-H), 7.72 (d, 1H, <i>J</i> = 8.4 Hz, 6-H), 7.85 (d, 1H, <i>J</i> = 8.4 Hz, 6'''-H), 7.90 (s, 1H, -NH), 8.09 (s, 1H, 2-H), 8.22 (d, 2H, <i>J</i> = 7.2 Hz, 2'',6''-H)
5p	70.48 (70.41)	5.08 (5.17)	3.30 (3.42)	1.02, 1.08 (t, t, 6H, <i>J</i> = 7.2 Hz, CH ₃), 3.24 (m, 4H, CH ₂), 6.94 (t, 1H, <i>J</i> = 8.4 Hz, 3'-H), 6.98 (t, 1H, <i>J</i> = 8.4 Hz, 5'-H), 7.43 (d, 1H, <i>J</i> = 8.4 Hz, 5-H), 7.43 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.50 (t, 3H, <i>J</i> = 7.6 Hz, 2,3'',5''-H), 7.58 (d, 1H, <i>J</i> = 8.0 Hz, 6-H), 7.64 (t, 1H, <i>J</i> = 8.0 Hz, 4''-H), 8.18 (d, 2H, <i>J</i> = 7.2 Hz, 2'',6''-H)
5q	66.37 (66.51)	5.30 (5.13)	9.16 (9.31)	2.20 (s, 3H, -CH ₃), 2.28 (t, 4H, <i>J</i> = 4.2 Hz, 3'''-CH ₂), 3.38, 3.720 (t, t, 4H, <i>J</i> = 4.8 Hz, 2'''-CH ₂), 6.94 (t, 1H, <i>J</i> = 8.0 Hz, 3'-H), 6.97 (t, 1H, <i>J</i> = 8.0 Hz, 5'-H), 7.42 (d, 1H, <i>J</i> = 8.4 Hz, 5-H), 7.42 (q, 1H, <i>J</i> = 8.0 Hz, 6'-H), 7.49 (s, 1H, 2-H), 7.51 (t, 2H, <i>J</i> = 8.0 Hz, 3'',5''-H), 7.60 (d, 1H, <i>J</i> = 8.0 Hz, 6-H), 7.65 (t, 1H, <i>J</i> = 7.6 Hz, 4''-H), 8.18 (d, 2H, <i>J</i> = 8.0, 2''-H);
5r	64.92 (64.98)	4.78 (4.73)	4.97 (5.05)	3.21 (s, 6H, N-CH ₃), 6.93 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.95 (d, 1H, <i>J</i> = 8.5 Hz, 5'-CH), 7.10 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.36 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.46 (d, 1H, <i>J</i> = 8.5 Hz, 6-CH), 7.50 (s, 1H, 2-CH), 10.08 (s, 1H, OH)
5s	66.80 (66.87)	5.69 (5.61)	4.52 (4.59)	1.31 (t, 6H, <i>J</i> = 7.0 Hz, -CH ₃), 3.59 (q, 4H, <i>J</i> = 7.0 Hz, N-CH ₂), 6.92 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.96 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.10 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.36 (q, 1H, <i>J</i> = 8.0 Hz, 6'-CH), 7.45 (d, 1H, <i>J</i> = 8.5 Hz, 6-CH), 7.49 (s, 1H, 2-CH), 10.08 (s, 1H, OH)
5t	64.98 (65.05)	5.56 (5.46)	8.38 (8.43)	2.35 (s, 3H, -CH ₃), 2.50 (t, 4H, <i>J</i> = 5.0 Hz, 3'',5''-CH ₂), 3.80 (t, 4H, <i>J</i> = 5.0 Hz, 2'',6''-CH ₂), 6.92 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.96 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.09 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.36 (q, 1H, <i>J</i> = 8.5 Hz, 6'-CH), 7.43 (s, 1H, 2-CH), 7.47 (d, 1H, <i>J</i> = 8.5 Hz, 6-CH), 9.74 (s, 1H, OH)
5u	65.81 (65.88)	5.91 (5.82)	8.02 (8.09)	1.12 (t, 3H, <i>J</i> = 7.0 Hz, -CH ₃), 2.48 (q, 2H, <i>J</i> = 7.0 Hz, -CH ₂), 2.53 (t, 4H, <i>J</i> = 5.0 Hz, 3'',5''-CH ₂), 3.81 (t, 4H, <i>J</i> = 5.0 Hz, 2'',6''-CH ₂), 6.92 (t, 1H, <i>J</i> = 8.5 Hz, 3'-CH), 6.96 (d, 1H, <i>J</i> = 8.0 Hz, 5'-CH), 7.09 (d, 1H, <i>J</i> = 8.5 Hz, 5-CH), 7.35 (q, 1H, <i>J</i> = 8.5 Hz, 6'-CH), 7.44 (s, 1H, 2-CH), 7.45 (d, 1H, <i>J</i> = 8.5 Hz, 6-CH), 9.79 (s, 1H, OH)

Table 3

The anti-inflammatory and analgesic activities of compounds

Compound	The inhibitory effect on xylene induced mice ear edema		The inhibitory effect on acetic acid induced mice writhing	
	Dose/(mg kg ⁻¹)	Inhibition/%	Dose/(mg kg ⁻¹)	Inhibition/%
2a	40	13.65	40	0
2b	40	16.06	40	7.97
5a	40	48.83	40	29.87
5b	40	5.38	40	23.86
5c	40	0	40	15.63
5d	40	34.12	40	16.58
5e	40	23.16	40	8.90
5f	40	19.58	40	12.30
5g	40	12.78	40	18.96
5h	40	36.40	40	30.96
5i	40	41.37	40	11.74
5j	40	0	40	29.87
5k	40	22.03	40	13.20
5l	40	0	40	28.52
5m	40	68.86	40	47.50
5n	40	19.79	40	5.57
5o	40	21.40	40	20.79
5p	40	65.20	40	0
5q	40	27.47	40	88.33
1	40	39.85	40	8.33

and analgesic activity. So these results will provide a good idea to design and research the SAR in the future.

Fortunately, because of possessing an excellent anti-inflammatory activity and a good analgesic activity, **5m** may be a potential anti-inflammatory agent.

Table 4

The ED₅₀ of compound **5m**, **5p**, and **5q**

Compound	Test model	ED ₅₀ /(mg kg ⁻¹)
5m	On xylene induced mice ear edema	24.24
5p	On xylene induced mice ear edema	25.69
5m	On acetic acid induced mice writhing	43.79
5q	On acetic acid induced mice writhing	11.58

Acknowledgments

The authors thank the Opening Foundation of the Biochemical Engineering Key Discipline (20050105), Zhejiang, China, for financial support and the National Center for Drug Screening, Shanghai, China, for evaluation of anti-inflammatory and analgesic activity.

References and notes

- Fujisawa, H.; Fujiwara, T.; Takeuchi, Y.; Omata, K. *Chem. Pharm. Bull.* **2005**, *53*, 524.
- Bilecen, D.; Schulte, A. C.; Kaspar, A.; Kustermann, E.; Seelig, J.; Elverfeldt, D. V.; Scheffler, K. *NMR Biomed.* **2003**, *16*, 144.
- Betageri, G. V.; Nayernama, A.; Habib, M. J. *Int. J. Pharm. Adv.* **1996**, *1*, 310; Betageri, G. V.; Nayernama, A.; Habib, M. J. *Chem. Abstr.* **1995**, *124*, 193178.
- Green, N. S.; Palaninathan, S. K.; Sacchettini, J. C.; Kelly, J. W. *J. Am. Chem. Soc.* **2003**, *125*, 13404.
- Adamski-werner, S. L.; Palaninathan, S. K.; Sacchettini, J. C.; Kelly, J. W. *J. Med. Chem.* **2004**, *47*, 355.
- Funk, L. A.; Mary, M. C.; Kung, P. P.; Meng, J. J.; Zhou, J. Z. WO Patent 2006,117,669, 2006.
- Yu, C. X.; Xu, L. WO Patent 2008,012,603, 2008.
- Kucukguzel, G.; Kocatepe, A.; Clercq, E. D.; Sahin, F.; Gulluce, M. *Eur. J. Med. Chem.* **2006**, *41*, 353.

9. Hung, D. Y.; Mellick, G. D.; Anissimov, Y. G.; Weiss, M.; Roberts, M. S. *J. Pharm. Sci.* **1998**, *87*, 943.
10. Zhong, G. X.; Zhao, K. CN Patent 101,029,010A, 2007.
11. Zhong, G. X.; Zhao, K. CN Patent 101,033,198A, 2007.
12. All the chemicals and solvents were analytical reagent and used as received, unless otherwise stated. Melting points were taken on XRC-1 apparatus and are uncorrected. IR spectra were measured on a Bio-Rad FTS-40 spectrometer (KBr); ^1H NMR spectra were recorded on a Bruker AC-80 spectrometer (400 MHz) or a Bruker AC-100 spectrometer (500 MHz) using TMS as the internal standard in CDCl_3 . MS spectra were run on an HP5989B instrument.
13. General procedure for the synthesis of compound **5**. A solution of diflunisal **1** (1 equiv) and pyridine (2 equiv) in CHCl_3 was stirred for 30 min to protect the carboxyl group by forming a salt (mp 126–129 °C after being separated). To this reaction mixture was added dropwise a solution of RCOCl (1 equiv) in chloroform at room temperature, and the reaction was allowed to proceed with stirring for an additional about 3 h (monitored by TLC). After that 1 M HCl was used to precipitate, filtrate, wash and dry to afford compound **2**. It was observed that insufficient amount of pyridine would decrease the yield. Compound **2a** ($\text{R} = \text{CH}_3$): yield 98%, white crystalline solid, mp 161–165 °C; MS m/z : 292 $[\text{M}]^+$ (2), 250 (49), 232 (100), 204 (27), 175 (23), 156 (7). Compounds **2b** ($\text{R} = \text{C}_6\text{H}_5$): yield 95%, white crystalline solid, mp 167–172 °C; IR (KBr, cm^{-1}): 3444, 1738, 1689, 1267, 1210, 706; ^1H NMR (500 MHz, CDCl_3): δ (ppm): 6.96 (t, 1H, $J = 8.0$ Hz, 3'-H), 7.00 (t, 1H, $J = 8.0$ Hz, 5'-H), 7.35 (d, 1H, $J = 7.5$ Hz, 5-H), 7.46 (q, 1H, $J = 8.0$ Hz, 6'-H), 7.50 (t, 2H, $J = 8.0$ Hz, 3'', 5''-H), 7.65 (t, 1H, $J = 7.6$ Hz, 4''-H), 7.80 (d, 1H, $J = 8.0$ Hz, 6-H), 8.21 (d, 2H, $J = 7.5$ Hz, 2'', 6''-H), 8.25 (s, 1H, 2-H), 11.24 (s, 1H, -COOH); MS m/z : 354 $[\text{M}]^+$ (2), 232 (20), 175 (4), 151 (2), 122 (4), 105 (100), 77 (29), 51 (5). A mixture of compound **2** and SOCl_2 (2 equiv) in CH_2Cl_2 was refluxed for 4 h prior to work up to give compound **3**. The compounds **5** were prepared by reaction of **3** (1 equiv) with substituted amines **4** (2 equiv) in CH_2Cl_2 at room temperature for 3 h, in 72–89% total yields based on the diflunisal. Compound **5a**: MS m/z : 305 $[\text{M}]^+$ (2), 263 (77), 245 (22), 233 (23), 232 (100), 204 (24), 175 (21), 151 (9). Compound **5b**: MS m/z : 319 $[\text{M}]^+$ (3), 277 (73), 259 (20), 233 (28), 232 (100), 204 (17), 175 (17), 151 (6). Compound **5c**: IR (KBr, cm^{-1}): 3069, 2937, 2852, 1767, 1633, 846, 818; MS m/z : 373 $[\text{M}]^+$ (5), 332 (15), 249 (28), 232 (100), 204 (16), 175 (18), 151 (12), 98 (17). Compound **5d**: IR (KBr, cm^{-1}): 3069, 2863, 1765, 1634, 865, 833; MS m/z : 381 $[\text{M}]^+$ (3), 339 (77), 232 (18), 204 (10), 175 (11), 151 (5), 106 (18), 91 (100). Compound **5e**: MS m/z : 367 $[\text{M}]^+$ (4), 325 (32), 233 (39), 232 (12), 204 (8), 175 (16), 151 (10), 93 (100). Compound **5f**: IR (KBr, cm^{-1}): 3204, 2860, 1760, 1640, 875, 846; MS m/z : 381 $[\text{M}]^+$ (2), 339 (27), 233 (28), 204 (4), 177 (10), 175 (7), 151 (7), 107 (100). Compound **5g**: MS m/z : 381 $[\text{M}]^+$ (3), 339 (16), 233 (21), 204 (5), 177 (6), 175 (6), 151 (5), 107 (100). Compound **5h**: MS m/z : 381 $[\text{M}]^+$ (4), 339 (15), 233 (25), 204 (4), 177 (6), 175 (5), 151 (5), 107 (100). Compound **5i**: MS m/z : 401 $[\text{M}]^+$ (2), 359 (35), 233 (66), 232 (39), 204 (16), 175 (26), 151 (18), 127 (100). Compound **5j**: MS m/z : 401 $[\text{M}]^+$ (2), 359 (22), 233 (63), 232 (14), 204 (8), 175 (16), 151 (10), 127 (100). Compound **5k**: IR (KBr, cm^{-1}): 3199, 2860, 1763, 1641, 864, 857; MS m/z : 395 $[\text{M}]^+$ (4), 353 (29), 233 (34), 204 (4), 177 (11), 175 (8), 151 (8), 121 (100). Compound **5l**: IR (KBr, cm^{-1}): 2858, 1762, 1639, 1613, 1255, 847, 807; MS m/z : 361 $[\text{M}]^+$ (3), 319 (98), 275 (35), 233 (100), 204 (18), 175 (29), 151 (23), 86 (81). Compound **5m**: IR (KBr, cm^{-1}): 3392, 1719, 1665, 1611, 1481, 1272, 1213, 709; MS m/z : 367 $[\text{M}]^+$ (2), 245 (123), 232 (2), 204 (2), 175 (4), 151 (2), 105 (100), 77 (24). Compound **5n**: IR (KBr, cm^{-1}): 3335, 1745, 1647, 1597, 1442, 1265, 1200, 707; MS m/z : 429 $[\text{M}]^+$ (1), 337 (4), 307 (5), 232 (2), 180 (5), 106 (8), 105 (100), 77 (23). Compound **5o**: IR (KBr, cm^{-1}): 3445, 1736, 1644, 1602, 1487, 1272, 1213, 706; MS m/z : 443 $[\text{M}]^+$ (1), 337 (3), 321 (5), 194 (3), 175 (3), 106 (10), 105 (100), 77 (40). Compound **5p**: IR (KBr, cm^{-1}): 1739, 1633, 1471, 1266, 1211, 710; MS m/z : 409 $[\text{M}]^+$ (5), 337 (2), 175 (4), 151 (2), 106 (8), 105 (100), 77 (20), 72 (18). Compound **5q**: IR (KBr, cm^{-1}): 1735, 1634, 1462, 1260, 1208, 708; MS m/z : 436 $[\text{M}]^+$ (7), 379 (54), 337 (7), 233 (8), 175 (6), 106 (8), 105 (100), 77 (39). Compound **5r**: MS m/z : 277 $[\text{M}]^+$ (100), 276 (30), 233 (56), 232 (82), 204 (27), 175 (30), 151 (23), 44 (37). Compound **5s**: MS m/z : 305 $[\text{M}]^+$ (65), 304 (43), 232 (57), 204 (21), 175 (23), 151 (33), 72 (100), 58 (76). Compound **5t**: MS m/z : 332 $[\text{M}]^+$ (33), 275 (7), 233 (18), 204 (5), 151 (11), 99 (22), 83 (18), 70 (100). Compound **5u**: MS m/z : 346 $[\text{M}]^+$ (39), 275 (9), 233 (33), 204 (6), 175 (8), 151 (15), 99 (19), 84 (100).
14. Zhong, G. X.; Li, J.; Zheng, R. H.; Zhao, K. *Acta Crystallogr. E* **2007**, *63*, o4583.
15. Brooks, R. R.; Bonk, K. R.; Decker, G. E.; Miller, K. E. *Agents Actions* **1985**, *16*, 369.
16. Graziella, B. EP Patent 0,126,287, 1984.