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

Guang-Xiang Zhong [a,*], Lu-Lu Chen [a], Hai-Bo Li [b], Fu-Jin Liu [a], Jin-Qing Hu [a], Wei-Xiao Hu [a]

[a] College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310032, PR China
[b] Nantong Center for Disease Control and Prevention, Nantong 226007, PR China

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ABSTRACT

To discover the new medicinal activity, the structure of diflunisal has been modified. Forty amide derivatives of diflunisal were synthesized starting from diflunisal in three steps. Their inhibition growth rate of human lung cancer cell (*A549*) and human endometrial adenocarcinoma cell (*Ishikawa*) in vitro was evaluated. The preliminary assay results showed that compounds **6j**, **7o** and **8c** exhibited good anti-tumor activities and excellent selectivity for the *Ishikawa* cell, may be potential anti-tumor agents.

Fluorine-containing drugs, such as tegadifur,[1,2] flutamide,[3,4] ciprofloxacin,[5,6] have been attractive for their special properties. 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.[7,8] Recently, the modifications of the chemical structure of **1** were studied. For example, 3-(1,3-dihydro-2*H*-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.[9] 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 dilunisal.[10] Some changes in the carboxyl group of **1** also showed good antimycobacterial, antiviral and antimicrobial activities.[11] Roberts found that *O*-aryl esters of **1**, especially lipophilic esters, possess large permeability surface area and tissue distribution value.[12] Zhong discovered that some amide derivatives of **1** exhibited potent anti-inflammatory and analgesic activity.[13,14]

Our group has studied the synthesis and the SAR of fluorine-containing anti-tumor drugs, and patented that some amides of fluorine-containing benzoic acid possessed good anti-tumor activity.[15] Recent, we discovered that some amide derivatives of **1** have good anti-tumor activities.

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 40 amide derivatives of diflunisal, *O*-acetyldiflunisal and *O*-benzoyldiflunisal, **5–8,** and evaluated their anti-tumor activity. The 36 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 carboxlic acid gave desired product **5–7**. Their preparations were published and the structures of compounds were identified by IR, ^{1}H NMR, MS and elemental analysis.[13,14] Other 4 amides of compound **8** were synthesized from 67% to 83% in total yield. The synthetic route is shown in Scheme 2. The structures of compounds were identified by ^{1}H NMR, MS and elemental analysis.[16] The crystal structure of **6a** was determined by X-ray.[17]

The anti-tumor activities of these compounds (**5–8**) to inhibit the growth of human lung cancer cell *A549* and human endometrial adenocarcinoma cell *Ishikawa* in vitro were evaluated. The Cisplation,[18,19] 15663-27-1, a registered anti-tumor drug, was used as a positive control. The results of the evaluation of anti-tumor activity are summarized on Table 1.

From Table 1, for the *A549* cell it could be found that there are two compounds, **7n** and **8a**, IC_{50} 8.36 and 9.01 μmol L^{-1}, which

* Corresponding author. Tel.: +86 571 88320722; fax: +86 571 88320557.
E-mail address: drzhonggx@sohu.com (G.-X. Zhong).

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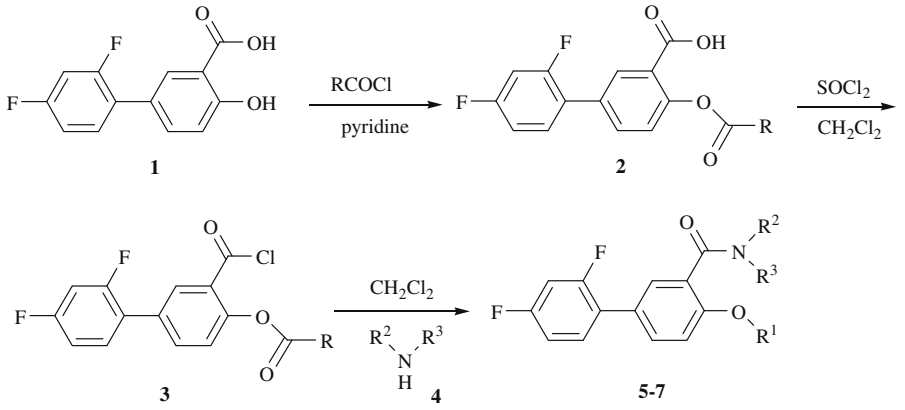
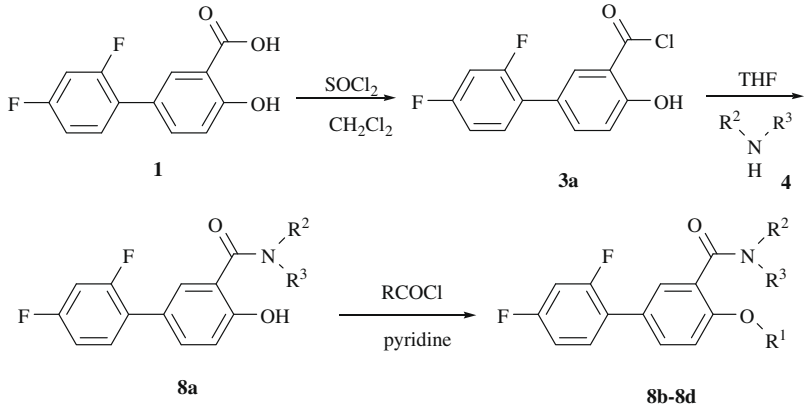


Scheme 1. Route of synthesis for compounds **5**–**7**.



Scheme 2. Route of synthesis for compound **8**.

possesses anti-tumor activity almost as same as that of Cisplation, IC_{50} 8.73. And for the *Ishikawa* cell it could be found that there are 17 compounds, which possesses anti-tumor activity superior to that of Cisplation, IC_{50} 12.20. Especially for **6c**, **6j**, **6l**, **7n**, **7o**, **8c** and **8d**, their IC_{50} are 0.94, 0.35, 0.47, 0.13, 0.69, 0.09 and 0.39 μmol L^{-1}, respectively, and the selectivity are 21, 290, 63, 64, 248, 348 and 52 relatively. Because of possessing excellent anti-tumor activities and good selectivity for the *Ishikawa* cell, **6j, 7o** and **8c** may be potential anti-tumor agents.

Additionally, it indicates that the substituted groups on amide could affect the activity. It was found that the compounds with N-aryl group are better than that with alkyl group in anti-tumor activity both amide derivatives of *O*-acetyldiflunisal and *O*-benzoyldiflunisal. For the amide derivatives of *O*-benzoyldiflunisal, it was discovered that the compounds with such electron-donating group (CH_3 group) seems to benefit as increase as following: *ortho* > *para* > *meta*, and the compounds with electro-withdrawing group (Cl group) is *meta* > *para* > *ortho*. Comparing **7h** with **7j**, **7k** and **7i** with **7l**, the electron-donating group (CH_3 group) reduces the anti-tumor activity for the *Ishikawa*. Comparing **7h** with **7m**, **7n** and **7o**, the electro-withdrawing group (Cl group) increases the anti-tumor activity for the *Ishikawa*. But for the amide derivatives of *O*-acetyldiflunisal, it was discovered that the compounds with such electron-donating group (CH_3 group) seems to benefit as increase as following: *meta* > *para* > *ortho*, with electro-withdrawing group (Cl group) is *ortho* > *para* > *meta*, and the electro-withdrawing group (Cl group) increases the anti-tumor activity for the *Ishikawa*. For the amide derivatives of diflunisal it was discovered that the anti-tumor activities are not good. From **6j**, **6l**, **7n** and **7o**, it was discovered that the electro-withdrawing group in amides is necessary.

To confirm the contribution of the electro-withdrawing group in *N*-group of amides, the compounds **8a**–**8c** were synthesized and evaluated. It was discovered that all three compounds possess good anti-tumor activities for the *Ishikawa* cell. Especially for compound **8c**, its IC_{50} is 0.09 μmol L^{-1} and the selectivity is 348 for the *Ishikawa* cell. It was proved that the electro-withdrawing group in amides will enhance the anti-tumor activity for the *Ishikawa*. Because of possessing excellent anti-tumor activity and selectivity for the *Ishikawa* cell, **8c** may be a really potential anti-tumor agent.

To research the contribution of the electro-withdrawing group in *O*-group of diflunisal, the compound **8d** synthesized and evaluated. It was found that anti-tumor activity or selectivity for the *Ishikawa* cell of **8d** is superior to that of **8a** and **8b**, but second to that of **8c**.

On the whole, the SAR is very complex and it needs more data to find the relationship. But it seems a trend that the substituted groups on the amide could affect on the anti-tumor activity. So these results will provide a good idea to design and research the SAR in the future.

Table 1
IC_{50} to inhibition growth of cancer cells for compounds (**5**–**8**)

Compound	R^1	R^2	R^3	In vitro inhibition (IC_{50}, μmol L^{-1})		Selectivity[a]
				A549	*Ishikawa*	
5a	H	CH_3	CH_3	>360	>360	/
5b	H	CH_2CH_3	CH_2CH_3	159.96	47.39	3.38
5c	H	4-Methylpiperazin-1-yl		229.91	16.22	14.2
5d	H	4-Ethylpiperazin-1-yl		75.18	2.45	30.6
5e	H	Imidazol-1-yl		120.76	27.60	4.37
6a	$COCH_3$	H	CH_3	178.59	97.75	1.83
6b	$COCH_3$	H	CH_2CH_3	130.70	70.65	1.85
6c	$COCH_3$	H	Cyclohexyl	20.49	0.94	21.9
6d	$COCH_3$	H	$CH_2C_6H_5$	42.64	81.18	1.90
6e	$COCH_3$	H	C_6H_5	86.21	4.74	18.2
6f	$COCH_3$	H	*o*-$C_6H_4CH_3$	61.33	203.27	3.31
6g	$COCH_3$	H	*m*-$C_6H_4CH_3$	111.12	6.11	18.2
6h	$COCH_3$	H	*p*-$C_6H_4CH_3$	109.08	142.35	1.31
6i	$COCH_3$	H	2,5-$(CH_3)_2C_6H_3$	124.71	12.54	9.94
6j	$COCH_3$	H	*o*-C_6H_4Cl	101.12	0.35	290
6k	$COCH_3$	H	*m*-C_6H_4Cl	>250	>250	/
6l	$COCH_3$	H	*p*-C_6H_4Cl	30.70	0.47	63.9
6m	$COCH_3$	Morpholine-4-yl		191.95	114.57	1.68
7a	COC_6H_5	H	CH_3	192.92	130.15	1.48
7b	COC_6H_5	H	CH_2CH_3	178.80	122.48	1.46
7c	COC_6H_5	H	$CH_2CH_2CH_3$	103.01	14.72	7.00
7d	COC_6H_5	CH_3	CH_3	132.00	142.46	1.08
7e	COC_6H_5	CH_2CH_3	CH_2CH_3	11.85	37.46	3.16
7f	COC_6H_5	H	Cyclohexyl	22.41	2.00	11.2
7g	COC_6H_5	H	$CH_2C_6H_5$	78.16	56.94	1.37
7h	COC_6H_5	H	C_6H_5	57.08	7.50	7.61
7i	COC_6H_5	H	*o*-$C_6H_4CH_3$	58.72	1.87	31.3
7j	COC_6H_5	H	*m*-$C_6H_4CH_3$	86.57	17.39	5.04
7k	COC_6H_5	H	*p*-$C_6H_4CH_3$	94.01	11.88	7.91
7l	COC_6H_5	H	2,5-$(CH_3)_2C_6H_3$	53.21	18.32	2.90
7m	COC_6H_5	H	*o*-C_6H_4Cl	56.14	4.05	13.8
7n	COC_6H_5	H	*m*-C_6H_4Cl	8.36	0.13	64.7
7o	COC_6H_5	H	*p*-C_6H_4Cl	171.06	0.69	248
7p	COC_6H_5	H	*p*-$C_6H_4OCH_3$	29.67	63.03	2.12
7q	COC_6H_5	4-Methylpiperazin-1-yl		>230	103.47	/
7r	COC_6H_5	Morpholine-4-yl		>230	>230	/
8a	H	H	4-NO_2-3-$CF_3C_6H_3$	9.01	3.79	2.38
8b	$COCH_3$	H	4-NO_2-3-$CF_3C_6H_3$	24.59	3.02	8.14
8c	COC_6H_5	H	4-NO_2-3-$CF_3C_6H_3$	31.43	0.09	348
8d	*p*-$NO_2C_6H_5CO$	H	4-NO_2-3-$CF_3C_6H_3$	20.50	0.39	52.34
Cisplation	/	/		8.73	12.20	/

[a] Selectivity is equal to $IC_{50,max}/IC_{50,min}$ for the both tested cells.

Acknowledgments

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16. The ^1H NMR, MS and EA of compound **8**.
Compound **8a**: 83%, white solid, mp 175–176 °C (butanone); ^1H NMR (500 MHz, $CDCl_3$, δ ppm): 6.96 (t, 1H, *J* = 8.5 Hz, 3′-H), 6.99 (t, 1H, *J* = 8.5 Hz, 5′-H), 7.16 (d, 1H, *J* = 8.5 Hz, 5-H), 7.40 (q, 1H, *J* = 8.5 Hz, 6′-H), 7.63 (d, 1H, *J* = 8.0 Hz, 6-H), 7.68 (s, 1H, 2-H), 8.05 (d, 1H, *J* = 8.0 Hz, 5″-H), 8.09 (s, 1H, 2″-H), 8.11 (d, 1H, *J* = 8.0 Hz, 6″-H, 8.29 (s, 1H, –NH), 11.30 (s, 1H, –OH); EIMS: *m/z* (%) = 438(M^+, 12.93), 233(100), 232 (89.69), 204 (18.02), 177 (28.11), 151 (25.23), 63 (4.45), 53 (16.24); Anal. Calcd for $C_{20}H_{11}F_5N_2O_4$: C, 54.81; H, 2.53; N, 6.39. Found: C, 54.69; H, 2.48; N, 6.30.
Compound **8b**: 67%, white solid, mp 171–173 °C (butanone); ^1H NMR (500 MHz, $CDCl_3$, δ ppm): 2.40 (s, 3H, $-CH_3$), 6.97 (t, 1H, *J* = 8.0 Hz, 3′-H), 7.01 (t, 1H, *J* = 8.0 Hz, 5′-H), 7.30 (d, 1H, *J* = 7.5 Hz, 5-H), 7.44 (q, 1H, *J* = 8.5 Hz, 6′-H), 7.73 (d, 1H, *J* = 8.0 Hz, 6-H), 7.98 (s, 1H, 2-H), 8.04 (d, 1H, *J* = 8.0 Hz, 5″-H), 8.07 (d, 1H, *J* = 8.0 Hz, 6″-H), 8.08 (s, 1H, 2″-H), 8.47 (s, 1H, –NH); EIMS: *m/z* (%) = 480 (M^+, 0.90), 438 (24.85), 233 (95.36), 232 (100.00), 204 (16.55), 175 (21.00), 151 (13.30), 53 (8.11); Anal. Calcd for $C_{22}H_{13}F_5N_2O_5$: C, 55.01; H, 2.73; N, 5.83. Found: C, 54.92; H, 2.63; N, 5.75.
Compound **8c**: 73%, white solid, mp 197–199 °C (butanone); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 6.97 (t, 1H, *J* = 8.4 Hz, 3′-H), 7.02 (t, 1H, *J* = 8.0 Hz, 5′-H), 7.41 (d, 1H, *J* = 8.4 Hz, 5-H), 7.48 (q, 1H, *J* = 8.4 Hz, 6′-H), 7.60 (t, 2H, *J* = 8.0 Hz, 3‴,5‴-H), 7.71 (s, 1H, 2-H), 7.75 (t, 1H, *J* = 8.0 Hz, 4‴-H), 7.78 (d, 1H, *J* = 8.0 Hz, 6-H), 7.92 (d, 1H, *J* = 8.0 Hz, 5″-H), 8.02 (d, 1H, *J* = 8.0 Hz, 6″-H), 8.15 (s, 1H, 2″-H), 8.24 (d, 2H, *J* = 8.0 Hz, 2‴,6‴-H), 8.85 (s, 1H, –NH); EIMS: *m/z* (%) = 542 (M^+, 5.13), 391 (3.92), 337 (18.54), 232 (73.34), 204 (68.72), 176 (65.61), 175

(100.00), 156 (33.37); Anal. Calcd for $C_{27}H_{15}F_5N_2O_5$: C, 59.79; H, 2.79; N, 5.16. Found: C, 59.82; H, 2.83; N, 5.05.

Compound **8d**: 75%, white solid, mp 230–232 °C (acetone); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 6.99 (t, 1H, *J* = 8.5 Hz, 3′-H), 7.03 (t, 1H, *J* = 8.5 Hz, 5′-H), 7.43 (d, 1H, *J* = 8.0 Hz, 5-H), 7.47 (q, 1H, *J* = 8.5 Hz, 6′-H), 7.79 (d, 1H, *J* = 8.0 Hz, 6-H), 7.94 (s, 1H, 2″-H), 7.95 (d, 1H, *J* = 8.5 Hz, 5″-H), 8.00 (d, 1H, *J* = 8.5 Hz, 6″-H), 8.29 (s, 1H, –NH), 8.39 (s, 4H, 2‴,3‴,5‴,6‴-H); EIMS: *m/z* (%) = 587 (M^+, 1.15), 420 (23.52), 382 (12.35), 232 (42.80), 204 (16.83), 175 (13.56), 150 (100.00), 120 (43.25); Anal. Calcd for $C_{27}H_{14}F_5N_3O_7$: C, 55.21; H, 2.40; N, 7.15. Found: C, 55.13; H, 2.35; N, 7.06.

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